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Volume 41 August, 1951 Number 8

Phytopathology

AN INTERNATIONAL JOURNAL

Official Organ of

The American Phytopathological Society

Contents

A preliminary report on the response of virus-infected Montmorency cherry trees to nitrogen fertilizer ..... JOHN C. CAIN AND K. G. PARKER 661
The destruction of Armillaria mellea in citrus soils ..... DONALD E. BLISS 665
Effect of various soil amendments on the inoculum potential of the Verticillium wilt fungus ..... STEPHEN WILHELM 684
Inheritance of resistance to spot blotch in barley seedlings. DEANE C. ARNY 691
The insect vector relationships of the filaree red-leaf virus, with special reference to a latent-period difference between nymphs and adults in Macrosiphum geranicola (Lambers) ..... CHRIS W. ANDERSON 699
Control of decay in prepackaged spinach ..... B. A. FRIEDMAN 709
Physiological races of the Crotalaria wilt Fusarium. JOANNE K. ARMSTRONG AND G. M. ARMSTRONG 714
Tests of cranberry varieties and seedlings for resistance to the leafhopper vector of false-blossom disease ..... R. B. WILCOX 722
Leaf rust of wheat, Puccinia rubigovera tritici, in Uruguay. CELIA S. BOASSO AND MOSES N. LEVINE 736
The occurrence of Phytophthora cinnamomi in the soil under pine stands in the Southeast ..... W. A. CAMPBELL 742
A new technique for incubating seed corn in cold soil for disease tests. PAUL E. HOPPE 747
Effect of temperature on growth and pathogenicity of Sclerotinia trifoliorum ..... K. W. KREITLOW AND V. G. SPRAGUE 752
Corn seedlings as test plants for the sugar-cane mosaic virus. A. S. COSTA AND M. P. PENTEADO 758
Phytopathological Notes:
Longevity of the red-node virus in beans. W. D. THOMAS, JR., AND R. W. GRAHAM 764
Bronze leaf of coconut palm in the Dominican Republic and Venezuela ..... R. CIFERRI 765
Red rot of sisal in Venezuela ..... R. CIFERRI 766

Subscription price: $10.00 per year, domestic and foreign

Office of Publication

Corner North Queen Street and McGovern Avenue, Lancaster, Pennsylvania

Entered at the postoffice in Lancaster, Pennsylvania, as second-class matter. Acceptance for mailing at the special rate of postage provided for in paragraph (d-2) Section 34.40, P. L. & R. of 1948. Authorized May 12, 1921.

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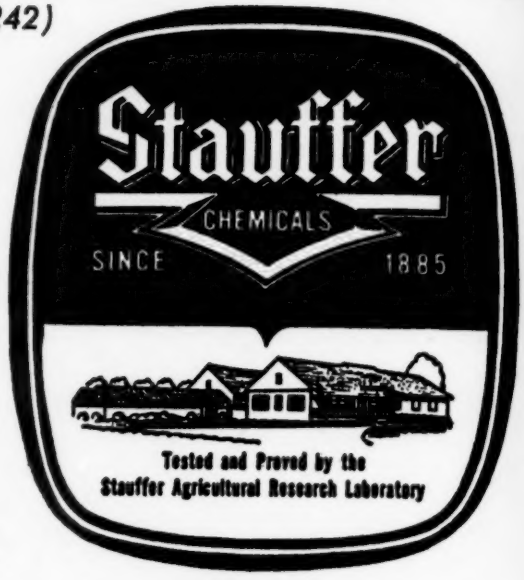

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A PRELIMINARY REPORT ON THE RESPONSE OF VIRUS-INFECTED MONTMORENCY CHERRY TREES TO NITROGEN FERTILIZER[1]

JOHN C. CAIN AND K. G. PARKER

(Accepted for publication December 12, 1950)

The results reported in this paper were obtained from an experiment to study the response of Montmorency cherries growing in sod to nitrogen fertilizer. Continuous sod culture was being tested for erosion control.

MATERIALS AND METHODS

The experimental orchard was located on the grounds of the New York State Agricultural Experiment Station, at Geneva, New York. The soil is Ontario loam on gently sloping terrain, fairly well drained. In 1942 the orchard was seeded to several different kinds of grass and legume sod to test the performance of these permanent sod crops under orchard conditions. One tree row was seeded to each kind of sod from center to center.

In the spring of 1947 these trees, then 13 years old, were in rather poor vigor and producing below normal. The orchard was divided into four equal blocks so that each block contained all of the different kinds of sod. Two of the blocks received each year 15 lb. ammonium sulfate per tree and the other two blocks 5 lb. per tree, applied to the soil around the tree about 2 weeks before bloom. The sod cover was mowed once or twice each year in early summer, depending on its rate of growth. Yield records have been kept on each tree since the trees began to bear. Measurements of trunk circumference and terminal shoot growth, made on 20 shoots per tree selected at random, have been made each year since fertilizer treatments were begun in 1947.

In the summer of 1947 the orchard was surveyed for two virus diseases, cherry yellows and ring spot. All trees without visible symptoms were indexed[2] to determine the presence or absence of the viruses. All trees contained the ring-spot virus and many of those having no visible symptoms had the cherry-yellows virus in the bud.

In 1949 all trees were graded into four classes for symptoms of yellows: 1) none, no visible yellows symptoms in 1949; 2) light, no yellow leaves but trees having typical growth habit of yellows-infected trees; 3) moderate, few yellow leaves; and 4) severe, many yellow leaves. Thus, comparisons were made among groups of trees having three degrees of cherry-yellows symptoms and those with no visible symptoms; however, in some of the latter group cherry yellows was present in the transmission tests. All trees

[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 828, May 4, 1950.

[2] Young Elberta peach shoots and Lovell peach seedlings were budded with cherry buds in late summer. The new peach growth the following spring had diagnostic symptoms if the cherry bud contained the virus.

had ring spot. Percentage fruit set and spur formation were measured in 1949 by counting flowers and spurs on two branches about 1 in. in diameter on each tree. The fruit on these branches was again counted shortly before harvest time.

RESULTS

Average data for all virus groups at the two levels of nitrogen fertilization are in table 1. Each fertilizer block had some trees from all virus

TABLE 1.—*The effect of nitrogen on yield and growth of sour cherries infected with cherry-yellows virus*

Virus rating[a]	Nitrogen[b]	No. of trees	Av. yield 1943–1946 (lb./tree)	Relative yield[c]			Percentage fruit set, 1949[d]	Spurs[e]	Shoot growth 1946 (mm./shoot)	Relative shoot growth[f]	
				1947	1948	1949				1947	1948
1, none	15	22	34	213	86	326	24	7.5	91	142	152
	5	21	44	183	55	247	21	6.8	93	89	104
	Av.	43	39	196	69	282	22	7.2	92	116	128
2, light	15	13	26	184	104	304	18	4.8	103	138	159
	5	14	36	146	39	190	9	2.8	93	83	117
	Av.	27	31	161	65	236	13	3.7	98	111	138
3, moderate	15	15	32	132	75	189	8	1.8	95	113	182
	5	11	38	115	47	127	8	0.5	91	76	116
	Av.	26	34	124	63	160	8	1.3	93	97	155
4, severe	15	1	52	98	42	0	1	0.0	67	105	237
	5	5	40	88	68	78	2	0.0	98	70	121
	Av.	6	42	90	63	62	2	0.0	93	74	134
Av.	15	51	32	179	85	275	18	5.1	95	131	163
	5	51	40	151	51	189	13	3.5	93	83	112

[a] 1, no visible symptoms; 2, growth habit typical for yellows-infected tree, no yellow leaves; 3, some yellow leaves; 4, many yellow leaves.
[b] Pounds per tree of ammonium sulfate annually in early spring since 1947.
[c] Average yield from 1943 to 1946 equals 100.
[d] Percentage of flowers setting fruit.
[e] Number of spurs formed since 1947 per 1000 flowers in 1949.
[f] 1946 shoot growth equals 100.

groups; however, only one severely affected tree received the high nitrogen and this tree declined rapidly and produced no fruit in 1949. All yields were low in 1948 because of frost injury at blossom time.

The data indicate a progressive increase from year to year in the yield differences among the groups of trees of different virus ratings. The average yield in 1943–1946 was 31 to 42 lb. per tree, with no appreciable differences among the groups. Yields in 1949, in terms of the 1943–1946 average on the same trees, ranged from 62 per cent on severely affected trees to 282 per cent on trees without visible symptoms in 1949. This indicates that the severely affected trees are declining rapidly in productivity, and those with milder yellows symptoms are not increasing as much as are symptomless ones. These results are in agreement with the findings of Keitt and Clayton (3) and of Moore (6), that the yield reduction in yellows-infected trees increases progressively during the years after symptoms first appear.

Both percentage fruit set and number of spurs produced decreased progressively as the severity of yellows increased, these factors largely accounting for the decrease in production. Extra nitrogen fertilizer has increased spur formation, percentage fruit set, and yield in all trees.

The virus condition apparently had no effect on the length of terminal shoot growth, which was greatly increased in all cases by nitrogen fertilization.

DISCUSSION

Keitt and Clayton (3) and Berkeley and Willison (1) described a reduction in fruiting spur production on yellows-infected trees. It is evident that the cherry-yellows virus causes a reduction in fruit yield of Montmorency cherries, probably through its effect on spur formation and percentage fruit set. The type of growth resulting when terminal extension continues with a reduced formation of spurs and lateral branches is commonly associated with trees infected with cherry yellows. The lateral buds on these shoots are largely flower buds which set a few fruits the second year and are completely barren thereafter. In contrast, on vigorous healthy shoots one or more of the basal buds are vegetative, forming spurs the second year and a fruit cluster the third year. These spurs then remain productive for several years.

It seems probable from the evidence in this paper that extra nitrogen has stimulated somewhat the vegetative condition of the yellows-infected tree. The formation of spurs is increased, resulting in more fruiting capacity on older wood which is normally reduced in yellows-infected trees.

Rasmussen and Cation (7) reported no influence on the development of the yellow-leaf symptoms when they applied various fertilizer materials, including nitrogen. Hildebrand (2) treated virus-infected trees by nitrogen fertilization and pruning and concluded that neither treatment corrects the condition. In his experiment all virus-infected trees were treated, with those not infected left untreated, and neither nitrogen nor pruning completely overcame the effect of the virus on yields. However, his experiment could not have shown any benefit less than complete recovery. The data presented here agree with Hildebrand's conclusion; nevertheless, it is believed that the extra nitrogen fertilizer has been beneficial in all cases.

Exactly how the virus has decreased fruit set is not known. Possibly the viability of the pollen is impaired or other flower parts are defective. Further study of the problem is contemplated.

Yellows-infected trees tend to bloom somewhat later than do healthy trees (3), and frost injury may have coincided with the incidence of yellows. However, an examination of the orchard plan reveals no evidence to support this. Keitt and Moore (4) and Mills (5) found that the lower the temperature while leaves are developing, the more virus yellows symptoms appear. It is possible that variations in temperature from tree to tree caused more virus symptoms and impaired pollen germination on some

trees, thus producing the effects noted on fruit set and yield. However, since nitrogen improved fruit set in all virus classes, local temperature variations within the orchard probably were not influential in this study.

SUMMARY

Evidence is presented from one orchard growing in sod that the yellows virus disease of Montmorency cherries causes reductions in yield, largely through reduced percentage of flowers setting fruit and less spur formation. A comparison of trees having received 15 lb. ammonium sulfate annually for 3 years with those receiving 5 lb. at different degrees of virus severity shows some improvement in growth, fruit set, and yield. Although this treatment has not completely overcome the effect of the virus in any case, it is suggested that the productivity of yellows-infected trees may be increased with extra nitrogen fertilization.

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THE DESTRUCTION OF ARMILLARIA MELLEA IN CITRUS SOILS[1]

DONALD E. BLISS[2]

(Accepted for publication January 22, 1951)

Armillaria root rot, considered the most important root disease of citrus in California, is widely distributed and kills many trees every year, all the common citrus rootstocks being somewhat susceptible. Destruction of the causal organism, *Armillaria mellea* (Vahl) Quel., by means of chemical soil treatment is one of the principal methods of controlling the disease; it is practicable, especially in treating small infested areas, and is desirable because of high land values and the imminent danger that the pathogen will spread to other areas.

Partial sterilization of soil with carbon disulfide, a method first employed in Europe by Girard (8) and Oberlin (12), was recommended by Horne (10) for destruction of *A. mellea* in California soils as early as 1914. Later investigations on dosage, means of application, and effectiveness were reported by Fawcett (7), Thomas and Lawyer (14), and Bliss (5).

Results of 15 years of field and laboratory experiments are recorded in the present paper: preliminary experiments on soil disinfestation with nine soil fumigants, experiments with carbon disulfide in two citrus orchards, and laboratory studies on *A. mellea* in pure and mixed cultures. Additional light is thrown on one of the basic principles involved in the destruction of *A. mellea* by means of partial sterilization of the soil.

SOIL DISINFESTATION

Preliminary trials in a lathhouse. Soil disinfestation studies in two series were initiated in 1935 to compare the efficiency of various fungicides and to determine the effect of such treatments on the soil for subsequent planting. Segments of citrus roots 1) naturally infected with *A. mellea* and 2) artificially infected by pure cultures were buried in fine sandy loam in galvanized iron containers 33 cm. in diameter and 58 cm. deep. As the cans were filled with air-dry soil and tamped, the root segments were laid horizontally at different depths ranging from 1 to 21 in. Large holes in the bottom of the containers had been covered with pieces of tile but not plugged. The soil surface was covered with two thicknesses of waxed paper and a 1-cm. layer of soil, but was not sealed against the escape of fumigating gases.

In the first series of experiments, four chemicals were used. For 1 sq. ft.

[1] Paper No. 672, University of California Citrus Experiment Station, Riverside, California.

[2] Plant Pathologist in the Experiment Station. The writer is indebted to the late H. S. Fawcett, and to Martin R. Huberty, C. L. Shear, James P. Martin, and Ellis F. Darley for helpful advice; to Elbert B. Griffith and C. W. Robinson for providing experimental test plots; to Calvin E. Bream, Robert O. Bream, A. G. Salter, D. M. Spencer, and the late William Picker; and to L. L. Huillier, W. W. McCoy, Robert J. Drake, Wesley D. Wilbur, and W. P. Sallee for assistance.

of surface area, the basic dosages were: carbon disulfide, 14.2 ml. (0.5 liq. oz.); formalin, 70.4 ml. in 6.9 l. water (1 per cent); tetrachlorethane, 6.7 ml. (100 p.p.m. dry weight of soil); and chloropicrin, 4.7 ml. These dosages were doubled for a second treatment. The chemicals were poured into holes made by driving a metal rod to a depth of 18 in. at the center of each container. These holes were closed immediately and the soil was covered.

The root segments were taken up 31 days after treatment. Viability of the fungus was tested in the laboratory by means of tissue plantings on cornmeal agar (Table 1), two from each root segment. Living mycelium

TABLE 1.—*Viability of Armillaria mellea in segments of naturally infected citrus roots at several depths, 31 days after soil treatment with various fungicides (1935 experiment)*[a]

Fungicide, and amount per sq. ft. of surface area	Container number	Root depth (inches)				
		1	6	11	16	21
None (control)	1	V	V	NV	V	NV
	2	V	V	V	V	NV–T
	3	V	V	V	V	V
	17	V	V	V	NV	NV
Carbon disulfide, 14.2 ml.	4	NV	NV	NV–T	NV	NV
	5	NV–T	NV–T	NV–T	NV–T	NV
Do 28.4 ml.	6	NV–T	NV–T	NV–T	NV–T	NV–T
	18	NV	NV	NV–T	NV–T	NV–T
Formalin,[b] 70.4 ml.	7	NV	NV–T	NV–T	NV–T	NV–T
	8	NV	NV	NV		NV
Do 140.8 ml.	9	NV	NV–T	NV–T	NV–T	NV–T
	10	NV	NV–T	NV–T	NV–T	NV–T
Tetrachlorethane, 6.7 ml.	11	NV	V	NV	V	NV–T
	12	NV	NV	NV	NV	NV
Do 13.3 ml.	13	NV	NV–T	NV–T	NV	
	19	NV–T	NV–T	NV	NV	NV
Chloropicrin, 4.7 ml.	14	NV	NV	NV–T	NV	NV–T
	15	NV	NV–T	NV	NV	NV–T
Do 9.3 ml.	16	NV	NV	NV	NV	NV–T
	20	NV	NV	NV	NV	NV

[a] V indicates viable Armillaria mycelium; NV indicates nonviable. (Each entry represents a separate test.) T indicates that *Trichoderma viride* developed in culture.

[b] Forty per cent formaldehyde by volume; applied as a drench in 6.9 l. of water.

of *A. mellea* developed in about three-fourths of the cultures from roots in untreated soil, but in none from roots in soil treated with carbon disulfide, formalin, or chloropicrin. The larger dose of tetrachlorethane was also effective in killing the fungus. *Trichoderma viride* Pers. ex Fries (1) developed in many cultures in which *A. mellea* was nonviable, and only in such cultures.

On the day that the root segments were taken up, 200 seeds of Henderson's Early Snowball variety of cauliflower were planted in each container to test the condition of the treated soil for subsequent planting. The results, reported by Bliss (3), indicate that treatment with chloropicrin improved the soil for subsequent planting, and that treatments with carbon disulfide

and with the basic dose of formalin also stimulated growth to a moderate degree. Tetrachlorethane and the higher dosage of formalin remained in the soil in such high concentrations, however, that the soil was not then favorable to plant growth.

In the second series of experiments, in 1936, two to four dosages of carbon disulfide, chloropicrin, chlorine, sulfur dioxide, ammonium hydroxide, ethylene oxide, and dichlorethyl ether were applied (Table 2). All chem-

TABLE 2.—*Viability of Armillaria mellea in artificially inoculated citrus roots at several depths, after soil treatment with various fungicides (1936 experiment)*[a]

Fungicide, and amount per sq. ft. of surface area	After 18 days At root depth (in inches)					After 40 days At root depth (in inches)				
	1	6	11	16	21	1	6	11	16	21
None (control)	V	V	V	NV–T	V	V	V	V	V	V
	V	V	V	NV–T	V	V	V	V	V	V
Carbon disulfide										
3.7 ml.	NV–T	NV–T	NV–T	NV–T	NV–T	NV–T	NV–T	NV–T	NV–T	NV–T
7.4 ml.	NV–T	NV–T	NV–T	V	NV–T	NV	NV–T	NV–T	NV–T	NV–T
14.8 ml.	V	NV	NV	NV	V	NV–T	NV–T	NV–T	NV–T	NV–T
Chloropicrin										
1.3 ml.	NV	NV	NV	NV–T	NV–T	NV–T	NV–T	NV–T	NV–T	NV–T
2.5 ml.	NV	NV–T	NV	NV	NV	NV–T	NV–T	NV–T	NV	NV–T
5.0 ml.	NV	NV	NV	NV	NV	.....	.....	.....	.....	.....
7.5 ml.	.....	.....	.....	.....	.....	NV	NV	NV	NV	NV
Chlorine										
81.0 gm.	NV–T	V	V	V	V	V	V	NV–T	V	NV–T
162.0 gm.	V	V	NV	NV	V	V	NV–T	NV	NV	V
323.0 gm.	NV	NV	NV	NV	NV	NV	NV	NV	NV	NV
Sulfur dioxide										
73.0 gm.	V	V	V	NV–T	V	NV–T	NV–T	V	NV–T	V
292.0 gm.	V	NV–T	NV	NV	NV	NV–T	NV–T	NV–T	NV	NV
Ammonium hydroxide[b]										
27.6 ml.	.....	.....	.....	.....	.....	V	NV–T	NV–T	NV–T	NV
110.4 ml.	V	V	V	V	V	.....	.....	.....	.....	.....
Ethylene oxide										
5.0 gm.	.....	.....	.....	.....	.....	NV–T	NV–T	NV	NV–T	NV
45.0 gm.	NV	NV	NV	NV	NV	.....	.....	.....	.....	.....
Dichlorethyl ether										
14.8 ml.	.....	.....	.....	.....	.....	NV–T	NV–T	NV–T	NV–T	NV–T
29.6 ml.	V	V	V	NV	NV	.....	.....	.....	.....	.....

[a] V indicates viable Armillaria mycelium; NV indicates nonviable. (Each entry represents a separate test.) T indicates that *Trichoderma viride* developed in culture.
[b] C.P.; 0.90 specific gravity; 28.5 per cent NH_3; applied in 28 l. of water.

icals except ammonium hydroxide were placed 18 in. deep and in the center of the container. The liquids were poured down a long-necked funnel, and the gases were delivered through a perforated metal tube. The given amounts of c.p. ammonium hydroxide were diluted in 28 l. water and applied as a drench to saturate the entire mass of soil.

Viability of the fungus in half the containers was tested 18 days, and in the other half 40 days, after treatment (Table 2). Viable mycelium of *A. mellea* was recovered in 18 of 20 tests from untreated roots, although in

four of these tests the mycelium of *T. viride* overran and killed the young, viable colonies of *A. mellea* in culture. *T. viride* also developed in the two cultures in which *A. mellea* was nonviable.

The results with carbon disulfide (Table 2), although not fully understood at the time, set a pattern for later experiments which revealed a new principle in soil disinfestation. Viable mycelium of *A. mellea* was recovered in three culture tests made 18 days after treatment, but in none of those made 40 days after treatment. In the 18-day tests the lowest dosage gave the best control of *A. mellea*. In these tests *T. viride* appeared in all the nonviable cultures from roots in soil which received the 3.7-ml. and 7.4-ml. doses, but not in those of the 14.8-ml. treatment. In the 40-day tests, *T. viride* developed in 14 of the 15 cultures.

No viable mycelium of Armillaria developed in any roots treated with chloropicrin or ethylene oxide. Trichoderma appeared in tissue cultures from roots receiving the lower dosages, especially in tests made 40 days after treatment. Results from treatment with dichlorethyl ether were similar to those from carbon disulfide. Chlorine and sulfur dioxide were not effective except at the higher dosages, and ammonium hydroxide was not effective at either dosage used.

Forty-six days after treatment, the soil in each container was planted, as in the 1935 tests, with 200 seeds of cauliflower. Differences in the appearance of the various lots of plants were very noticeable 50 days later (3). Chloropicrin was again outstanding in stimulating growth, besides increasing the percentage of emergence. Carbon disulfide seemed to stimulate growth, but the number of plants was reduced. Although ethylene oxide increased emergence, the plants were so crowded that the average fresh weight was only a little greater than that of the control. Emergence was much reduced where chlorine had been used. Results were also inferior with sulfur dioxide, ammonium hydroxide, and dichlorethyl ether.

Chloropicrin, carbon disulfide, and ethylene oxide, having given the most encouraging results in the preliminary experiments, were then considered on the basis of relative adaptability to field conditions. Ethylene oxide, a gas under ordinary conditions of temperature and pressure, was ruled out because no practicable method of application was known. (For the preliminary experiments, dosages of this fungicide had been weighed by difference on a solution balance.) Chloropicrin and carbon disulfide, being liquids, could be measured by volume in the field. Preliminary trials with these two chemicals were therefore made on naturally infected roots in the field. Whereas in open, sandy soils the effect of chloropicrin was lethal only within 12 to 24 in. vertically from the site of application, carbon disulfide killed to a depth of at least 60 in. Since viable mycelium of Armillaria had been found in citrus roots at 9-ft. depth, it was thought that the fungus could be killed more easily with carbon disulfide (CS_2) than with chloropicrin. For this reason, CS_2 was used exclusively in later experiments.

Field experiment at Azusa. The effectiveness of CS_2, in different amounts and at different soil depths, was tested at the Elbert B. Griffith Ranch at Azusa, California, during the winter of 1938–1939.

Healthy citrus roots had been cut in 7-in. lengths, placed in glass jars, sterilized, inoculated with *A. mellea*, and incubated for 4 months to permit the pathogen to penetrate the wood thoroughly and to form pseudosclerotia (6). Two circular pits, 6 ft. in diameter and 11 ft. deep, were dug between rows of large Valencia orange trees. Holes $2\frac{1}{2}$ in. in diameter and 38 in. long were driven horizontally into the walls of the pits by means of a large soil tube (Fig. 1). These holes were arranged in five series for each of the

FIG. 1. Soil-tube apparatus used in making holes $2\frac{1}{2}$ in. in diameter and 38 in. long in walls of a pit located between rows of large Valencia orange trees. These holes were made in vertical series at 1-ft. intervals, from 1 ft. to 10 ft. below the surface, for placement of citrus-root segments inoculated with *Armillaria mellea*. All buried root segments were retrieved later for use in culture tests. Photographed by D. M. Spencer.

two pits, those in each series being in a vertical row, at 1-ft. intervals from 1 ft. to 10 ft. below the surface. The inoculated root segments were then removed from the glass jars and labeled individually; one large root (1–$2\frac{1}{4}$ in. diam.) and one small root ($\frac{5}{8}$–$1\frac{3}{8}$ in. diam.) were inserted to the distal end of each hole, and soil was packed in solidly. Finally, the pits were filled with tamped soil.

The soil was of the Hanford series, predominantly coarse sandy loam, with gravelly strata at depths of 3 to 4 ft. and also below 9 ft. When the roots were buried, the soil moisture ranged from 4 to 13 per cent. After this, but before treatment, there were 6 inches of rain. The surface was protected by canvas, but some water entered about the edges, and at one place the moisture ranged from 10 to 21 per cent in the upper 6 ft. of soil.

To fumigate the soil, measured volumes of CS_2 were poured through a

long-necked funnel to the bottom of holes made with a 1-in. soil tube driven vertically to depths of 1, 2, or 6 ft. in the undisturbed soil outside the pits. The holes were filled with soil and tamped immediately after the fumigant had been placed. For all treatments the holes, 18 in. apart in rows 18 in. apart, were staggered to form a diamond pattern on the surface and were centered on the vertical series of buried root segments. Four charges of CS_2 (technical grade) were placed about the roots in series B, C, G, and H and eight charges in series D, F, and J. Series A, untreated, served as a control. Series E and I also were untreated, but were subject to the effect of the lateral and downward diffusion of the fumigant placed 3 ft. to one side, in treatments at series D and J, respectively. Because of the rainfall, no additional wetting of the surface soil was necessary to confine the fumigant.

The two pits were opened and all buried root segments retrieved 76 days after treatment. Viability of *A. mellea* in the roots was tested in the laboratory. Two tissue cultures were made from each root, one from wood below the cambium and the other from wood at the center of the root.

All roots in nontreated series A contained viable Armillaria but the fungus was nonviable in three of the 40 cultures. *Trichoderma viride* developed in these three cultures and overran or inhibited the development of Armillaria in seven others. In series B, where 52.6 ml. CS_2/sq. ft. (4 liq. oz./charge) had been applied at 6-ft. depth, Armillaria was viable only at 2-ft. depth. Trichoderma developed in 39 of the 40 cultures. In series C, where an equal dosage of CS_2 had been applied at 1-ft. depth, Armillaria was nonviable in the upper 8 ft. The application of 26.3 ml. CS_2/sq. ft. (2 liq. oz./charge) at 1-ft. depth (series D) killed Armillaria to 7-ft. depth, except in one root. A young colony of Armillaria from this root was quickly overrun and destroyed by Trichoderma. Nonviability of Armillaria in roots of series E below 3 ft. indicated a lateral and downward diffusion of CS_2 gas from the treatment of series D, 3 ft. to one side.

Doses of CS_2 from 26.3 ml. up to 105.2 ml./sq. ft. (2–8 liq. oz./charge) were used in treating series F, G, H, and J. No viable Armillaria was found in any tissue cultures from these series or from series I, although the fungus might reasonably have been viable in some roots of series H (26.3 ml./sq. ft. at 6-ft. depth) if it had been more widely separated from the other heavy treatments. The results demonstrated the common association of Trichoderma with Armillaria-infected wood after soil fumigation with CS_2. The toxicity of this fumigant, in the largest doses, to Trichoderma was shown in series F, G, and J. Trichoderma failed, in most cases, to develop from roots taken near the site at which the fumigant was applied.

Field experiment at Rivera. A second field experiment was made in C. W. Robinson's Valencia orange orchard at Rivera, California, during the winter of 1939–1940. The soil, also of the Hanford series, consisted of fine sandy loam or silt loam to a depth of 5 ft., a stratum of moist silty-clay loam in the sixth foot, and coarse sand below. Soil moisture was 9 to 24 per cent in the upper 5 ft., 27 per cent in the sixth foot, and 7 per cent in the

seventh. Two pits were excavated 20 ft. apart, and holes were made in vertical series in the side walls to a depth of 7 or 8 ft., as described above.

Naturally infected citrus-root segments, 12 in. long and 1 to 4 in. in diameter, were used singly in series B and I; whereas artificially inoculated root segments 7 in. long and $\frac{3}{4}$–$1\frac{1}{4}$ in. diameter (small) or $1\frac{1}{4}$–$2\frac{1}{4}$ in. diameter (large), as in the experiment at Azusa, were used in series A, C to H, and J, respectively. To isolate the various treatments, strips of heavy asphalt paper were placed against the sides of the pits before they were filled with

TABLE 3.—*Viability of Armillaria mellea in infected citrus roots at several depths, 98 days after soil treatment with carbon disulfide (1939 experiment, Rivera, California)*[a]

Series and treatment	Size of roots[b]	Root depth (in feet)							
		1	2	3	4	5	6	7	8
A. Control (no treatment)	Large	V	V	V	V	V	V	V	.....
	Small	V	V	V	V	V	NV–T	V	.....
B. Control (no treatment)	Large	V	V	V	V	NV–T	NV–T	V	.....
C. 19.7 ml. CS_2/sq. ft.; 1-ft depth	Large	NV–T	NV–T	NV–T	V	V	V	V	.....
	Small	NV–T	NV–T	NV–T	V	V	V	V	.....
D. 26.3 ml. CS_2/sq. ft.; 1-ft depth	Large	NV–T	NV–T	NV–T	NV–T	NV–T	V	V	.....
	Small	NV–T	NV–T	NV–T	NV–T	NV–T	V	V	.....
E. 39.5 ml. CS_2/sq. ft.; 1-ft depth	Large	NV–T	NV–T	NV–T	NV–T	NV–T	V	V	.....
	Small	NV–T	NV–T	NV–T	NV–T	NV–T	V	V	.....
F. Control (no treatment)	Large	V	V	V	V	V	NV–T	NV–T	NV–T
	Small	V	V	V	V	V	.....	NV–T	NV–T
G. 79.0 ml. CS_2/sq. ft.; 1-ft depth	Large	NV–T	NV–T	NV–T	NV–T	NV–T	NV–T	NV–T	NV–T
	Small	NV–T	NV–T	NV–T	NV–T	NV–T	.....	NV–T	NV–T
H. 26.3 ml. CS_2/sq. ft.; 1-ft. and 7-ft. depths	Large	NV–T	NV–T	NV–T	NV–T	NV–T	NV–T	NV–T	NV–T
	Small	NV–T	NV–T	NV–T	NV–T	NV–T	.....	NV–T	NV–T
I. 26.3 ml. CS_2/sq. ft.; 1-ft. and 7-ft. depths	Large	NV–T	NV–T	NV–T	NV–T	NV–T	NV–T	NV–T	NV–T
J. 79.0 ml. CS_2/sq. ft.; 7-ft. depth	Large	V	V	V	NV–T	NV–T	NV–T	NV–T	NV–T
	Small	V	V	NV–T	NV–T	NV–T	.....	NV–T	NV–T

[a] V indicates viable Armillaria mycelium; NV indicates nonviable. (Each entry represents two or three separate tests.) T indicates that *Trichoderma viride* developed in culture.

[b] Artificially inoculated roots were used in all series except B and I, in which naturally infected roots were used.

soil and tamped. The fumigation treatments were made as described above, except that the charges were applied at 1- and 7-ft. depths. To hold the fumigant in the soil, the top 4 in. of soil were moistened before and after treatment.

The root segments were taken up 98 days after fumigation. Two or three tissue cultures were made from each root, to test the viability of *A. mellea*. The fungus was viable in all but one root in series A, which had received no soil treatment (Table 3). *T. viride* developed in four cultures in which Armillaria was nonviable, and also overran and suppressed the growth of Armillaria in three others. The results from nontreated series B were erratic, probably because the condition of these naturally infected roots had not been uniform at the beginning. All treatments in series C, D,

and E had been applied at 1-ft. depth and differed only in the dosage of CS_2. In series C, 19.7 ml./sq. ft. (1.5 liq. oz./charge) killed Armillaria only in the upper 3 ft.; in series D, 26.3 ml./sq. ft. (2 liq. oz./charge) killed Armillaria in all roots in the upper 5 ft., but not below the moist clay-bearing stratum. Although in series E the dosage was 39.5 ml./sq. ft. (3 liq. oz./charge), the killing action extended downward only to the moist clay-bearing stratum, as in series D.

Series F to J, inclusive, were situated about the second pit. Armillaria was viable in all roots of nontreated series F from 1 to 5 ft., but not below (Table 3). No viable fungus remained at any depth in series G, where 79.0 ml. of CS_2 per sq. ft. (6 liq. oz./charge) had been applied at 1-ft. depth, or in series H and I with 26.3 ml. of CS_2 per sq. ft. (2 liq. oz./charge), applied at both 1-ft. and 7-ft. depths. In series J, however, with 79.0 ml. of the fumigant per sq. ft. at the 7-ft. level, Armillaria remained viable in the upper 3 ft. The nonviability of Armillaria at 6- to 8-ft. depths about the second pit (including nontreated series F) indicated that a rather widespread diffusion of the gas had occurred beneath the moist clay-bearing stratum. Fortunately, this effect had not extended to series A to E situated about the first pit, where all treatments had been made at 1-ft. level.

In this experiment, as in that at Azusa, *T. viride* developed in nearly all cultures where Armillaria had become nonviable (Table 3). This correlation was found, not only with fumigated roots, but also with a number of nonfumigated roots. Trichoderma also overran and suppressed the growth of Armillaria in certain other cultures. It is possible that Trichoderma may have been an air-borne contaminant in a few cultures, but for the most part this mold had been thoroughly established on the surface and within the wood of many roots before they were taken from the soil. Various other species of fungi and bacteria were also found in some cultures, but none of these occurred commonly, nor was their occurrence correlated with the nonviability of Armillaria.

ARMILLARIA AND TRICHODERMA IN MIXED CULTURE

The investigation then turned to a study of Trichoderma and its effect on the growth and viability of Armillaria in culture. First, an attempt was made to isolate Armillaria from mixtures of Armillaria and Trichoderma in agar cultures, but without success. The rapid-growing mycelium of Trichoderma overran and quickly suppressed Armillaria (2); it also produced a greenish yellow pigment which diffused through the agar and exerted a toxic effect on Armillaria similar to that on *Rhizoctonia solani* previously observed by Weindling (15). Moreover, the mycelium of Armillaria soon became apparently nonviable in the presence of Trichoderma.

A question then arose concerning the ability of Armillaria to develop naturally and retain its viability for long periods in tree roots when Trichoderma is present in the surrounding soil. The first experiment to test the longevity of Armillaria was begun December 8, 1937. Segments of citrus

roots 2.5–3.5 cm. in diameter and 31 cm. long were sterilized in large test tubes containing small amounts of bran and free moisture but no soil. The tubes were divided into three lots; these were inoculated, the first with *A. mellea* and the second with *T. viride;* in the third, inoculated with *A. mellea* and *T. viride,* Armillaria became established within the roots before Trichoderma was added. All tubes were held in a darkened room at 20°–30° C., and sterile water was added at intervals of 6 to 8 months. Tissue cultures were made from one root of each lot at approximately yearly intervals until 1946 (Table 4).

TABLE 4.—*Longevity of Armillaria mellea and Trichoderma viride in pure and mixed cultures from citrus-root segments*

Inoculum	Time of culture (months)	Fungi[a] recovered from cultures		
		Bark	Outer wood	Inner wood
A. mellea	14	A	A	A
	25	A	A	A
	38	A	A	A
	51	A	.....	A
	73	A & P	.....	A & P
	85	P	.....	A & P
	98	.....	P	P
T. viride	14	T	T	T
	25	T	T	T
	38	T	T	T
	51	T	.....	T
	73	T	.....	T
	85	T	.....	T
	98	T	T	T
A. mellea	14	T	A & P	A
and	25	T	A	A
T. viride	38	T	A	A & T
	51	T	A	A & T
	73	T	.....	A & T
	85	T	T	A, T & P
	98	T	T	A, T & P

[a] A, Armillaria; T, Trichoderma; P, Penicillium.

Armillaria, inoculated in pure culture, was recovered from the roots in all reisolations up to 85 months. Contamination by a bluish green *Penicillium* sp. was evident in these cultures after 73 months. Trichoderma, inoculated in pure culture, was reisolated without contamination over the entire period. In the third lot, Trichoderma was reisolated regularly from the bark and, after 38 months, occasionally from the wood, while Armillaria was reisolated in pure culture from the wood for 85 months. Armillaria was viable but was overrun by Penicillium after 98 months.

Under these conditions Armillaria remained viable within its pseudosclerotium for 7 years in spite of external contamination by Trichoderma during the full period, and by Penicillium during the latter part. Armillaria caused marked decomposition of the wood in the lower, moister ends of the root segments, whereas Trichoderma, alone, did not. Toward the

end of the period, Armillaria appeared to be losing its ability to prevent the invasion of the contaminating fungi through the pseudosclerotium. This weakening in vitality accompanied the exhaustion of food reserves and perhaps, also, the accumulation of toxic metabolic products. Trichoderma, however, showed no signs of weakening. Its greater longevity may have been due partly to spore formation.

Other experiments were made to study the longevity of Armillaria-infected citrus roots in moist, nonsterile soil containing Trichoderma. Artificially inoculated segments 3–4.5 cm. in diameter and 12 cm. long, buried in a citrus orchard at Riverside on December 27, 1939, contained viable mycelium of Armillaria after 76 months. Other artificially inoculated segments 5–9 cm. in diameter and 45 cm. long were buried in moist, nonsterile peat moss in galvanized iron containers on April 8, 1941. Most of these roots were viable when first exposed to the inoculum and, although bearing necrotic lesions, produced weak, leafy shoots for several years. Other roots had been killed by steaming prior to inoculation. Cultures were made from roots of both kinds after 82 months, and all contained viable mycelium of Armillaria. The toadstools of this fungus developed at various times from some of these roots. In a third test, sterile citrus-root segments 11–15 cm. in diameter and 23 cm. long were inoculated with Armillaria under pure-culture conditions on April 8, 1941; 6 months later they were transferred to moist, nonsterile soil in galvanized iron containers. One segment was taken up occasionally to test the viability of the fungus. Armillaria was still viable in a segment cultured 107 months after inoculation. The wood in this root was becoming soft and jellylike in some places, but the pseudosclerotium of Armillaria was still intact, and pure cultures of the fungus were obtained directly from the wood. Toadstools had formed from this root each winter for several years.

From these tests it is apparent that the mycelium of Armillaria, after becoming established in a citrus root at least 3 cm. in diameter, will remain viable 6 years or more in moist, nonsterilized soil containing Trichoderma. The period of viability is probably affected by the amount of food reserve within the pseudosclerotium.

THE ROLE OF TRICHODERMA IN SOIL DISINFESTATION

Three controlled experiments were made to test the role of Trichoderma in soil disinfestation of Armillaria. Similar methods were employed throughout. Citrus-root segments 3–4 cm. in diameter and 17 cm. long were steam-sterilized in 2-qt. glass jars plugged with cotton. A small amount of bran folded in cheesecloth had been tied about the lower end of each segment, and water added, before sterilization. Some roots were left sterile; others were inoculated with *A. mellea* (culture B-905). The fungus first became established in the bran (Fig. 2, A) and then, within 3 or 4 months, thoroughly invaded the roots (Fig. 2, B).

In the meantime, moist top soil from a citrus orchard at the University

of California Citrus Experiment Station was screened, thoroughly mixed, and placed in other 2-qt. glass jars. Some jars were untreated; others were steam-sterilized. The soil in certain jars was then inoculated with Trichoderma (culture B-952), and in one experiment the soil was also inoculated with *Fusarium solani* (culture B-984). Within 2 to 3 months, when the inoculum became established and when Armillaria had invaded the roots, the jars containing the roots were filled with soil in such a manner as to give various combinations of variables. Another incubation for about 3 weeks allowed the rhizomorphs of Armillaria to develop in the soil, and the soil-inhabiting fungi, including Trichoderma, to become established in or on the roots.

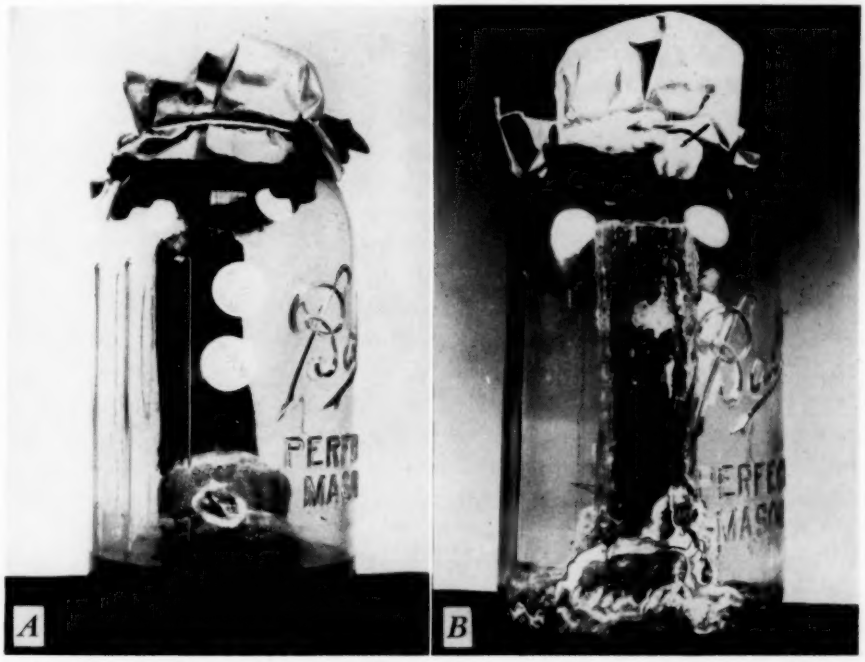

FIG. 2. Inoculation of citrus-root segments with *Armillaria mellea* in pure culture: A, young colony established on a small amount of bran folded in cheesecloth and tied to the root segment; B, root completely invaded by the fungus which has produced a pseudosclerotium about the fungus body. Photographed by W. D. Wilbur.

The jars were then divided equally in two similar groups. The first group remained untreated; the second was fumigated for 7 days in a large steel container. Technical grade CS_2 was allowed to evaporate near the top of the container, 59.1 ml. being used per 9 cu. ft. of air, or 2.32 p.p.m. Culture tests were made from some of the roots at the end of the 7-day fumigation. Other roots were allowed to stand undisturbed and without further fumigation for 24 days (31 days from the beginning of fumigation) before culture tests were made. Two or three tissue cultures were made from wood just beneath the bark and from the center of each root segment.

These cultures, on cornmeal agar slants, were incubated at 20° to 25° C. and observed at 2- or 3-day intervals for 1 month. Special effort was made to detect evidence of viability of Armillaria. The presence of Trichoderma was noted and, also, in the third experiment, the presence of Fusarium. Other fungi and bacteria found especially in nonsterilized soil were not usually identified.

Results of these culture tests may be summarized as follows: (a) No Armillaria was recovered from any root that had not been artificially inoculated with that fungus. (b) Nearly all sterilized roots in jars containing sterile soil or no soil gave sterile cultures. (c) Sterilized roots in jars of nonsterile soil became infested with numerous species of microorganisms of such soil-inhabiting groups as bacteria, Streptomyces, Penicillium, Mucor, and others.

Experiment 1. Air temperatures of 14° to 37° C. were recorded outside the fumatorium during the 7 days from July 26 to August 2, 1948, when the jars were fumigated. In culture tests made immediately after fumigation Armillaria was viable in all types of Armillaria-infected roots, regardless of soil pretreatment or fumigation treatment. In culture tests from other roots which had been undisturbed for 24 days following fumigation, Armillaria was nonviable in sterilized soil containing Trichoderma, both with and without fumigation, and in fumigated nonsterile (natural) soil. Armillaria was viable in nonfumigated natural soil. Trichoderma developed in most cultures in which Armillaria was nonviable. Armillaria was not destroyed by fumigation in the absence of Trichoderma. In jars where Trichoderma had been added to sterilized soil, or where natural soil (containing Trichoderma) had been fumigated, large masses of green Trichoderma spores were found in soil and on root surfaces 31 days after the beginning of fumigation. Whereas the viable mycelial fans of Armillaria were white, turgid, and of a loose, felty consistency, those that were nonviable were dull to greenish white or brownish and flaccid. Finally, spore masses of Trichoderma formed on the nonviable mycelial fans.

Experiment 2. The second experiment was like the first, except that it included one series of jars without soil. During the fumigation period, from February 21 to 28, 1949, air temperatures ranged from 9° to 31° C. The sterilized soil contained 7 per cent moisture; the nonsterile soil, 10 per cent. The roots had been inoculated with Armillaria for 119 days, the soil had been inoculated with Trichoderma for 70 days, and the soil and roots had been combined for 22 days prior to fumigation.

Culture tests made immediately after fumigation indicated that up to this time neither Armillaria nor Trichoderma had been killed by the fumigation (Table 5). Trichoderma was isolated, however, from Armillaria-infected wood both with and without fumigation. As in experiment 1, Armillaria was mostly nonviable in the series of tests with sterilized soil containing Trichoderma: the protective mechanism of Armillaria evidently broke down and Trichoderma developed profusely. In natural, nonsterile

soil, Armillaria was still viable in most roots, and Trichoderma had not become dominant in the soil microflora. In the jars containing sterilized soil, a white, diffuse mycelium growing from the Armillaria-infected roots was cultured directly from the soil and was identified as *A. mellea*. Such mycelium was not observed in the presence of competing organisms.

Important changes occurred when other culture tests were made 32 days after the beginning of fumigation (Table 5). No viable Armillaria was recovered from fumigated roots in nonsterile natural soil, although the fungus was still viable in similar, nonfumigated roots. This confirmed the results of many fumigation tests in the orchard. Trichoderma was cultured from

TABLE 5.—*Results of culture tests showing relation of Trichoderma viride to destruction of Armillaria mellea in citrus roots fumigated in soil with carbon disulfide (Experiment 2)*

Soil pretreatment and fumigation[a]	Viable fungi recovered in culture tests[b]			
	7 days after beginning of fumigation of root segments		32 days after beginning of fumigation of root segments	
	Sterilized	Sterilized and inoculated with Armillaria	Sterilized	Sterilized and inoculated with Armillaria
No soil, nonfumigated	2 –	6 A	2 –	6 A
Do, fumigated	2 –	6 A	2 –	6 A
Autoclaved, nonfumigated	2 –	6 A	2 –	6 A
Do, fumigated	2 –	6 A	2 –	7 A
Autoclaved and inoculated with Trichoderma, nonfumigated	2 T	9 T	2 T	8 T, 1 –
Do, fumigated	2 T	2 A, 5 T, 1 –	2 T	8 T, 1 –
No pretreatment (natural soil), nonfumigated	2 –	5 A, 2 T	2 –	8 A, 1 T
Do, fumigated	2 –	9 A	2 –	7 T, 2 –

[a] CS_2 was applied to root segments and soil in jars at the rate of 59.1 ml. per 9 cu ft., or 2.32 p.p.m. in air, for 7 days.

[b] –, sterile culture or unidentified organism; A, Armillaria; T, Trichoderma. The numbers represent separate tests.

nearly all roots where Armillaria was nonviable. Armillaria was viable, however, in the absence of Trichoderma, regardless of fumigation treatment; and it was nonviable in the presence of Trichoderma, even without fumigation.

These results indicate that Armillaria in citrus roots is destroyed by the antibiotic action of Trichoderma and not, usually, by the direct toxic effect of CS_2. Trichoderma can destroy Armillaria in citrus roots when competing organisms are not present, but in nonsterile natural soil Trichoderma does not become sufficiently dominant to destroy Armillaria until about 1 month after fumigation.

Experiment 3. This experiment was similar to the second, except that culture tests were made from all the roots 31 days after the beginning of

fumigation, the jars of all series contained soil, and *Fusarium solani* was introduced as a new variable. This species of Fusarium, commonly associated with citrus roots, was moderately antagonistic to Trichoderma when colonies of the two fungi were grown on agar plates. The possibility that Fusarium might prevent Trichoderma from becoming dominant in nonfumigated citrus soils was therefore tested.

During the fumigation period, from October 14 to 21, 1949, air temperatures ranged from 5° to 28° C., and soil-moisture percentages were approximately the same as in experiment 2. The roots had been inoculated with Armillaria for 94 days, the soil had been infested with Fusarium and Trichoderma for 42 days, and the soil and roots had been combined for 25 days prior to fumigation.

TABLE 6.—*Results from culture tests showing relation of Fusarium solani and Trichoderma viride to the destruction of Armillaria mellea in citrus roots fumigated in soil with carbon disulfide (experiment 3)*

Soil pretreatment and fumigation[a]	Fungi recovered 31 days after beginning of fumigation of root segments[b]	
	Sterilized	Sterilized and inoculated with Armillaria
Autoclaved, nonfumigated	2 –	7 A, 1 –
Do, fumigated	2 –	7 A, 1 –
Autoclaved and inoculated with Fusarium, nonfumigated	2 F	8 A
Do, fumigated	2 F	7 A, 1 –
Autoclaved and inoculated with Trichoderma, nonfumigated	2 T	1 A, 8 T
Do, fumigated	1 T, 1 –	8 T, 1 –
Autoclaved and inoculated with Fusarium and Trichoderma, nonfumigated	2 M	5 M, 1 F, 1 T, 3 –
Do, fumigated	2 F	3 M, 1 F, 2 T, 2 –
No pretreatment (natural soil), nonfumigated	3 F	**5 A, 2 T**, 1 –
Do, fumigated	2 F	8 T, 1 –
Natural soil inoculated with Fusarium and Trichoderma, nonfumigated	2 –	6 A, 1 T, 1 –
Do, fumigated	2 F	8 T

[a] CS_2 applied to root segments and soil in jars at the rate of 59.1 ml. per 9 cu. ft., or 2.32 p.p.m. in air, for 7 days.

[b] – indicates sterile culture or unidentified organism; A, Armillaria; T, Trichoderma; F, Fusarium; M, mixture of Fusarium and Trichoderma. The numbers represent separate tests.

The following observations were made 31 days after the beginning of fumigation, and just before making culture tests. In sterilized soil, there was greater development of diffuse mycelium and rhizomorphs of Armillaria in nonfumigated than in fumigated soil. Fusarium, alone, in sterilized soil had developed abundantly but had not checked the growth of Armillaria rhizomorphs. Trichoderma, alone, had developed profusely in sterilized soil and on root surfaces, and had definitely restricted the elonga-

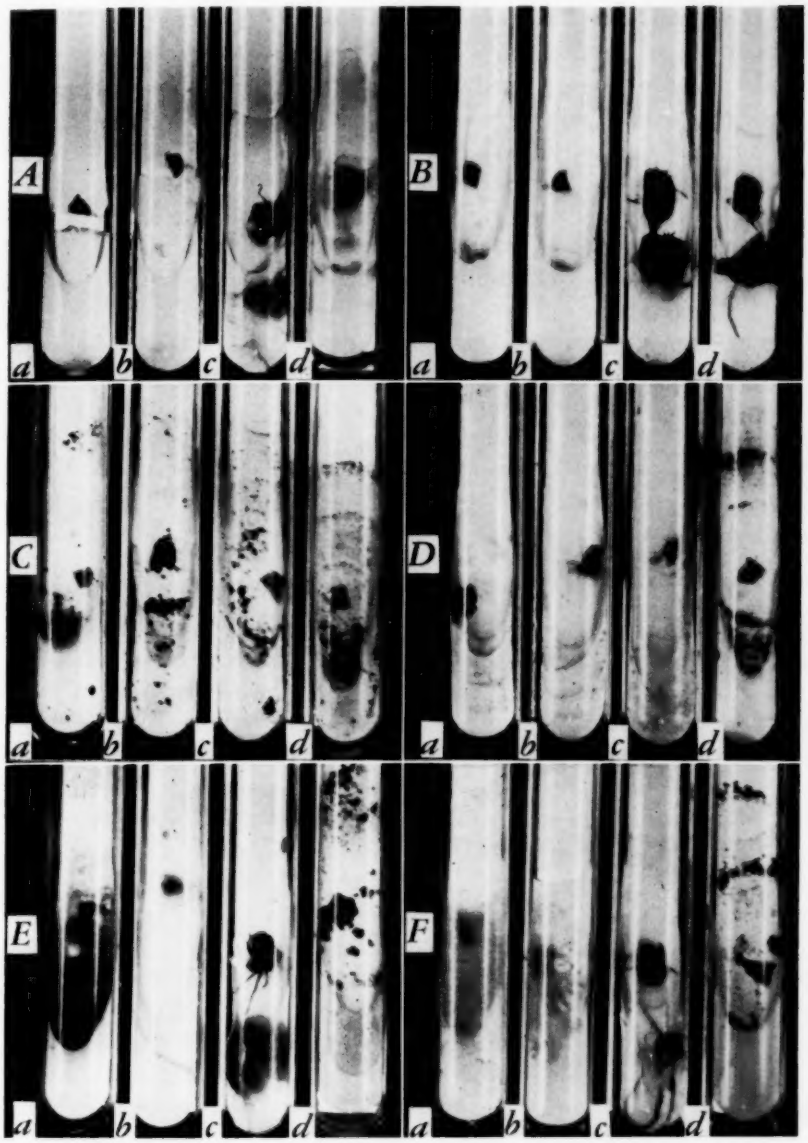

FIG. 3. Representative culture tests from experiment 3 (after 59 days), showing the role of Trichoderma in soil disinfestation of Armillaria in citrus roots. Each culture represents a separate root: A, from sterilized soil; B, from sterilized soil infested with *Fusarium solani;* C, from sterilized soil infested with *Trichoderma viride;* D, from sterilized soil infested with *F. solani* and *T. viride;* E, from nonsterilized soil; F, from nonsterilized soil infested with *F. solani* and *T. viride.* Cultures a and b of each group were from roots without Armillaria; c and d, from roots with Armillaria. Cultures a and c were from nonfumigated roots; b and d, from roots fumigated 7 days with carbon disulfide and held an additional 24 days before culturing. Armillaria may be distinguished in cultures A(c and d), B(c and d), E(c), and F(c) by its rhizomorphs. Trichoderma may be distinguished in cultures C(a, b, c, and d), D(d), E(d), and F(d) by the dark granular spore masses. Note that Armillaria was killed only in the presence of Trichoderma. In nonsterilized soil Armillaria was killed only after fumigation. Photographed by W. D. Wilbur.

tion of the Armillaria rhizomorphs. Little or no development of Armillaria had occurred in sterilized soil containing a mixture of Fusarium and Trichoderma. In nonsterilized soil, either with or without the addition of Fusarium and Trichoderma, many rhizomorphs of Armillaria had grown normally in nonfumigated, but not in fumigated, soil. Conversely, masses of Trichoderma spores had formed in the fumigated, but not in the nonfumigated, soil.

Results of culture tests show that Armillaria was generally nonviable in roots from fumigated or nonfumigated sterilized soil to which Trichoderma, alone, or Fusarium and Trichoderma had been added (Table 6). In the absence of Trichoderma, Armillaria was viable, and neither Fusarium alone nor fumigation affected viability where Trichoderma was lacking. In natural, nonsterilized soil, however, with or without addition of Fusarium and Trichoderma, Armillaria usually remained viable except where fumigated. The fact that Fusarium exerted little effect on the antibiotic action of Trichoderma indicates that it probably did not suppress Trichoderma in nonfumigated natural soil. Some typical cultures are shown in figure 3.

DISCUSSION

During the early phases of this work, it was believed that soil disinfestation of Armillaria was accomplished by the direct toxicity of various fumigants to the pathogen. Chloropicrin, ethylene oxide, and carbon disulfide applied in large doses seemed to kill Armillaria and other associated organisms by direct action. Armillaria was also killed with smaller doses where there was less destruction of associated organisms. A mild form of partial soil sterilization in which Armillaria was killed was considered superior to one in which most of the soil-inhabiting fungi and bacteria were killed.

In choosing a suitable fumigant for practical use, physical properties outweighed direct toxicity. Ethylene oxide, which has a boiling point of 14° C., was not suited for use in the field because no practical means of application had been developed. Chloropicrin, the most toxic of the group, had a relatively low vapor pressure, which made it unsatisfactory for use in deeply infested soils. CS_2, although relatively low in toxicity, was selected as being best suited for use against Armillaria because of its high vapor pressure (298 mm. at 20° C.) and its ability to penetrate deeply into soil. Other favorable factors included (a) low relative cost, (b) availability of self-measuring mechanical applicators and of large quantities of the chemical, and (c) effective use in the field for many years.

The almost constant association of Trichoderma with roots in which Armillaria had become nonviable was noted in all fumigation experiments, especially where CS_2 had been applied in small or moderate doses. At first this was considered to be coincidental, and it was thought that Trichoderma, being tolerant of the treatment, was merely one of the first soil-inhabiting molds to enter the roots after Armillaria had been killed by the fumigation.

As it became evident, however, that Armillaria could not be grown on agar in the presence of Trichoderma, or isolated from a mixed culture, the possibility of antibiotic activity was suggested. But how could Armillaria in infected roots remain viable for 6 to 9 years while buried in nonsterile soil containing Trichoderma and other soil inhabitants? This question was answered in part by Campbell (6), who discovered the protective and reproductive functions of the pseudosclerotium. Campbell found that the vegetative mycelium of Armillaria, after becoming established in the root or trunk of a suscept, develops a protective layer of thick-walled fungus cells, the pseudosclerotial covering, about the body of infected wood and the white mycelial fans in the cambium region. The rhizomorphs resulting from the apical growth of the pseudosclerotium are also covered by this protective layer.

From the present studies it is apparent that normally the pseudosclerotium resists attack by Trichoderma in the presence of other soil-inhabiting organisms. The longevity of Armillaria in the pseudosclerotial phase of its life cycle is determined largely by the extent of its food reserve and by other environmental conditions. If the normal biological equilibrium of the soil is disturbed, however, as by fumigation with CS_2, Trichoderma develops rapidly and overcomes the protective mechanism of Armillaria. After gaining entrance to the pseudosclerotium, Trichoderma requires only time to reach all parts of the fungus body and to kill the mycelium of Armillaria by antibiotic action. This action apparently extends to the center of the larger roots as effectively as to that of the smaller ones. More time may be required, but this reaction does not necessarily depend on the diffusion of the fumigant itself to the center of the root, as was formerly supposed.

There is still much to be learned regarding the biological effects of fumigation with CS_2 on citrus soils. Although this treatment, in heaviest doses, is fungicidal to both Armillaria and Trichoderma, it is only mildly fungistatic when applied at the usual rate and is perhaps somewhat stimulating to Trichoderma in small amounts (13). Since Trichoderma is widely distributed in citrus soils but is usually found only in small numbers (11), there is circumstantial evidence that its development, also, may be controlled by competing organisms.

Recent investigations emphasize the need for thorough fumigation of infested soil to destroy Armillaria. In tests by Thomas and Lawyer (14), low soil moisture was the most important single factor in fumigation with CS_2; when 45 ml. of CS_2 were applied 8–9 in. deep at 18-in. staggered intervals, Armillaria was killed to a depth of 60 in. and sometimes, with a 3- to 6-in. surface blanket of moistened soil, to 70 or 80 in. Hagan (9) found that compact soil layers, such as plowsoles, interfered seriously with the distribution of CS_2, but that moisture content affected the permeability of the soil to CS_2 vapor more than any other variable studied. Except in fine sand, permeability became negligible as moisture content approached field capacity.

The present studies indicate that in citrus orchards Armillaria was killed more effectively with 59.15-ml. charges at 18-in. staggered intervals (26.3 ml./sq. ft. or 302 gal./acre) than with 45-ml. charges. In permeable soil Armillaria was killed to a depth of 84 in. The fungus in deep-lying taproots was killed by applying four 177-ml. depth charges of CS_2 near the diseased tree, 4 ft. apart and 4–6 ft. deep (4). In one experiment, a moist clay-bearing stratum was practically impermeable to CS_2 vapor.

For effective destruction of Armillaria, it now appears necessary for the CS_2 fumes to penetrate all infested soil and to be held in sufficient concentration to allow Trichoderma to become dominant in the soil microflora. The antibiotic action of Trichoderma against Armillaria requires about 1 month after fumigation.

SUMMARY

Field and laboratory experiments over 15 years are reported. In preliminary tests the lethal effects of nine chemicals on Armillaria in citrus root segments were compared. The viability of Armillaria was determined by means of culture tests 18 to 40 days after the beginning of the treatment. Best results were obtained from the use of chloropicrin, carbon disulfide, and ethylene oxide.

Carbon disulfide, although relatively low in toxicity, was best suited for use against Armillaria in the orchard because of its high vapor pressure and its ability to penetrate deeply into soil. The other two fumigants presented problems of application: ethylene oxide, because of its low boiling point; chloropicrin, because of its low vapor pressure.

The effectiveness of CS_2 against Armillaria when applied in different amounts and at different depths was tested in two mature orange orchards. Before fumigation, artificially inoculated citrus root segments were placed in vertical series at intervals of 1 ft., from 1 ft. to 10 ft. below the soil surface. CS_2 was applied in holes at 18-in. staggered intervals. The viability of Armillaria was determined by means of culture tests 76 to 97 days after treatment. CS_2 at 26.3 ml. per sq. ft. (2 liq. oz./charge, or 302 gal./acre) at 1-ft. depth was most satisfactory, killing Armillaria to 7-ft. depth in coarse sandy soil, and to 5-ft. depth in fine sandy loam. In the second orchard, a moist, clay-bearing stratum in the sixth foot stopped the downward movement of the gas. Armillaria below this level was killed by additional charges placed beneath the stratum.

In these experiments *Trichoderma viride* developed in nearly all cultures in which Armillaria had become nonviable. Trichoderma overran and killed Armillaria when the two were planted on the same agar plate, and Armillaria was not recovered from mixed cultures.

Longevity tests indicated, however, that the mycelium of Armillaria, after becoming established in citrus roots at least 3 cm. in diameter, remained viable for 6 years or longer in moist, nonsterilized soil containing Trichoderma. This was attributed to the protection provided by the pseudosclerotial covering of the fungus body.

Under biologically controlled conditions, Trichoderma profoundly affected soil disinfestation of Armillaria. Fumigation with CS_2 for 7 days, at an initial concentration of 2.32 p.p.m. in air, or 59.1 ml./9 cu. ft., in combination with sterilized soil, did not kill Armillaria in citrus root segments. Trichoderma, however, when grown in sterilized soil, killed the fungus even without fumigation. In roots in nonsterilized soil containing Trichoderma and many other kinds of soil-inhabiting organisms, Armillaria remained viable where not fumigated, but was killed when fumigated. The destruction of Armillaria by the antibiotic action of Trichoderma began by the end of the 7-day fumigation period, but was not complete until about 24 days later. Although in some of the earlier experiments Armillaria was apparently killed by the direct toxic action of CS_2 when applied in very large doses, it is concluded that when the fumigant is applied to orchard soil at the usual rate (302 gal. per acre) the destruction of Armillaria is due primarily to the antibiotic action of Trichoderma. The reason for the rapid development of Trichoderma in fumigated orchard soil is not known.

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EFFECT OF VARIOUS SOIL AMENDMENTS ON THE INOCULUM POTENTIAL OF THE VERTICILLIUM WILT FUNGUS

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(Accepted for publication March 17, 1951)

In a previous paper (26) a technique was described for determining quantitatively the inoculum potential of *Verticillium albo-atrum* Rke. & Bert. in soils. Inoculum potential was expressed as the infection index, which is defined as the percentage of indicator plants planted in a consistent number and pattern per unit volume of soil from which Verticillium could be isolated after a growing period of 6 to 8 weeks. The unit volume of soil was that required to fill an 8-in. pot, or approximately 4 liters. The indicator plant was the Bonny Best tomato. The term inoculum potential, used already in several senses (7, 8, 27), is used here to mean the amount of inoculum per unit volume of soil reflecting the infective power of the soil. Data in support of this usage as applied to Verticillium wilt studies were presented earlier (26). Additional data are presented in this paper.

Evidence is accumulating that certain soil-borne plant pathogens can be effectively suppressed during decomposition processes when the soil is free from living host tissues. The saprophytic microflora of the soil is assumed to play an important role in this suppression. Supporting this view have been the results of studies on the control of potato scab (15), Phymatotrichum root rot of cotton (2, 11, 16), root rot of wheat (24), root-knot nematode (14), *Rhizoctonia solani* (1, 21), and the root-rot complex of strawberry (6, 9). Although decomposition of amendments tends more commonly to suppress the soil pathogen, instances of the reverse action have been reported (9, 21, 23). The amendment may effect control of the disease by improving nutrition of the suscept, thus augmenting its resistance. Wheat in the experiments of Clark and others (2, 22) remained free from the take-all disease in soils high in nitrogen and phosphorus, notwithstanding the presence of the pathogen. Control of the southern Sclerotium rot of sugar beets was also effected by amendments which enriched the soil in nitrogen (13).

DETERMINATION OF INOCULUM POTENTIAL BY USE OF THE INFECTION INDEX

The soil used was Verticillium-infested clay-loam, previously cropped with tomatoes and known from tests to give a high infection index. The infection index was determined on the basis of 100 indicator Bonny Best tomato plants planted in ten 8-in. clay pots representing a quantity of approximately 1.5 cu. ft. of soil for each amendment. The amendments and amounts used are listed in table 1. All materials were mixed thoroughly with the soil by hand and the pots were filled, placed on a greenhouse bench, and watered. Subsequent mixings were made after 2 and 4 weeks, then 10 small Bonny Best tomato seedlings were transplanted into each pot. After

TABLE 1.—*Representative tests showing inoculum potential of Verticillium albo-atrum as affected by soil amendments. Infection index is the percentage of plants infected with Verticillium out of 100 indicator plants*

Time of year	Amendment	Infection index[a] Amount of amendment used: percentage by weight of air-dry soil				
		0.0	0.01	0.1	0.5	1.0
Oct.–Jan.	Check—soil untreated	62				
	Cottonseed meal					32
	Dithane Z-78		20	0		
	Spergon		59	47		
	Fermate		15	0		
Nov.–Feb.	Check—soil untreated	72				
	Barley straw			69		22*
	Blood meal			70		0
	Fish meal			82*		0*
	Gypsum				72	
	Cottonseed meal with 0.5 per cent gypsum		32			14
Feb.–May	Check—soil untreated	81*				
	Conifer wood sawdust			90*		95
	Conifer wood sawdust with 0.5 per cent ammonium sulfate			10		14
	Ammonium sulfate				8*	
	Cottonseed meal					33
	Cottonseed meal with 0.01 per cent Dithane Z-78					79
	Cottonseed meal with 0.01 per cent Fermate					33
	Cottonseed meal with 1.0 per cent conifer wood sawdust					15
	Fermate		27			
	Dithane Z-78		12			
	Cow manure, screened		76			77

[a] The infection rating of each of the 10 pots of a series is given in table 2 for the infection index ratings marked with an asterisk (*).

a growing period of 8 weeks each plant was examined for symptoms and cultured near the base of the stem to determine whether or not it was infected with the wilt Verticillium.

RESULTS

Addition of certain amendments to Verticillium-infested soil in some cases brought about a considerable reduction in its infective power, and in others gave no reduction (Table 1). These relations are indicated in the infection index columns where the lower the infection index, the greater is the reduction in the inoculum potential. All the data upon which the infection index values are based are given in table 2 for a few representative tests. They indicate the rather uniform infection obtained in the 10 pots of a test.

Amendments high in nitrogen (blood meal, fish meal, and ammonium sulfate) have given the greatest reductions in the infection index values. One per cent of blood meal or of fish meal reduced the index to 0, and 0.5

TABLE 2.—*Infection ratings of the 10 pots of a test showing representative data upon which the infection indices, marked with an asterisk (*) in table 1, were based*

Amendment	Infection rating of individual pots										Infection index
	1	2	3	4	5	6	7	8	9	10	
Check—soil untreated	8	10	8	7	5	7	7	10	10	9	81
Barley straw, 1.0 per cent	0	3	2	1	4	2	2	3	1	4	22
Fish meal, 1.0 per cent	0	0	0	0	0	0	0	0	0	0	0
Fish meal, 0.1 per cent	9	7	8	7	10	7	10	8	7	9	82
Conifer wood sawdust, 0.1 per cent	10	8	10	7	10	9	7	9	10	10	90
Ammonium sulfate, 0.5 per cent	0	1	1	2	0	0	1	1	1	1	8
Conifer wood sawdust, 0.1 per cent, with ammonium sulfate, 0.5 per cent	0	0	1	0	0	2	2	0	2	3	10

per cent of ammonium sulfate reduced it to 8, as compared to an index of approximately 75 for the untreated soil. One per cent of barley straw gave a very substantial reduction. Additions of cottonseed meal with gypsum or with conifer wood sawdust gave greater reductions than were obtained with cottonseed meal alone. Gypsum and conifer wood sawdust applied alone gave no reduction. The combination of 0.01 per cent of Dithane Z-78 and 1.0 per cent of cottonseed meal cancelled the salutary effects of the individual components. The combination of 0.01 per cent Fermate with 1.0 per cent cottonseed meal gave approximately the same degree of control that each gave when used separately. Conifer wood sawdust applied with ammonium sulfate did not appreciably affect the salutary effects of ammonium sulfate. The nitrogen-rich amendments which gave a high degree of control when applied at the rate of 1.0 per cent gave no measurable control at 0.1 per cent.

RELATION OF DECOMPOSITION TIME OF SOIL AMENDMENTS TO SUPPRESSION OF VERTICILLIUM

To determine the relation of decomposition time to suppression of Verticillium, the amendments listed in table 3 were added to Verticillium-in-

TABLE 3.—*The effect of decomposition time of soil amendments on the infection index of Verticillium*

Amendment, and percentage used	Infection index, determined on the basis of 50 indicator plants		
	Decomposition time, in weeks		
	1	3	8
Check, soil untreated	96	98	94
Chopped alfalfa, 2.5	66	84	22
Cottonseed meal, 2.5	....*	16	6
Blood meal, 2.5	....	....	2
Dithane Z-78, 0.1	8	2	6

* Plants failed to grow in these pots, probably because of toxic products of decomposition.

fested field soil in five 8-in. pots in the amounts indicated, and the pots were planted to tomato seedlings after 1-, 3-, and 8-week decomposition periods respectively. The plants were examined and cultured after a growing period of 8 weeks.

The results (Table 3) indicate a general downward trend with time in the infection indices of the soils treated with organic amendments. This would seem to indicate that either the products of decomposition, the associated microfloras and faunas, or both, are involved in bringing about these effects. The untreated soil did not change appreciably. The infection indices of the Dithane Z-78 treated soil were reduced abruptly and remained at a low level throughout the experiment. This would seem to indicate that the action of the Dithane against Verticillium is directly fungicidal. The blood meal in these experiments did not give quite the same degree of control as in previous tests. This indicates the variability encountered in this type of experiment.

RELATION BETWEEN INFECTION INDEX AND INOCULUM POTENTIAL

To determine further (26) the relation between infection index and inoculum potential, field-infested soil was diluted with varying proportions of partially sterilized (chloropicrin-fumigated) compost soil in ratios of

TABLE 4.—*Relation between infection index and inoculum potential of Verticillium in different dilution ratios of infested and noninfested soil*

Dilution ratio		Infection ratings of the 10 pots of a dilution series										
Infested soil	Noninfested soil	Pot numbers										Infection index
		1	2	3	4	5	6	7	8	9	10	
0	all	0	0	0	0	0	0	0	0	0	0	0
1	1000	0	0	0	0	0	0	0	0	0	0	0
1	500	0	0	1	0	0	0	0	0	0	0	1
1	100	0	1	0	0	1	0	0	0	0	0	2
1	50	1	1	1	1	0	0	0	1	0	0	5
1	25	1	3	3	3	2	3	1	2	1	3	22
1	10	4	4	4	5	6	4	5	5	4	4	45
1	5	4	6	7	4	7	5	4	5	8	9	59
all	0	8	10	10	6	10	6	9	9	6	8	82

1:1000, 1:500, 1:100, 1:50, 1:25, 1:10, and 1:5. Each dilution was replicated 10 times, the infection index obtained for each dilution series accordingly being based upon 100 indicator plants.

The results (Table 4) show that Verticillium was not detected in the 1:1000 dilution as indicated by the infection index of 0, but was recovered in the lesser dilutions. The infection index increased progressively as the proportion of infested soil in the dilution increased. When plotted, this gives a slowly rising curve up to the 1:50 dilution. Here the curve starts to rise rapidly in a nearly straight line as would be expected for simple dilutions. In many of the pots giving low infection indices, i.e., ratings of

1 or 2, Verticillium was most commonly confined to a single vascular bundle per stem. In pots with high indices, i.e., 7 to 10, Verticillium usually was present in all of the vascular bundles. It is not to be assumed that pots of soil rated 0 are entirely free from the Verticillium wilt fungus, because a second planting to indicator plants may yield some infection.

DISCUSSION AND CONCLUSIONS

Soil amendments rich in nitrogen were consistently effective in bringing about a substantial reduction in the inoculum potential of *V. albo-atrum* in soil. The reduction was roughly proportional to the nitrogen content of the amendment. For instance, approximately the same degree of control was obtained by 2.5 per cent cottonseed meal (Table 3), 1 per cent fish meal, 1 per cent blood meal, and 0.5 per cent ammonium sulfate. The actual N content of each ranged only from approximately 0.1 to 0.13 per cent. Assuming these results to apply to a field condition, control of wilt by soil amendment would require the addition of approximately 0.075 lb. of actual N per cu. ft. of soil, or greater than 1.5 tons per acre foot. These data may explain in part why field attempts to control Verticillium wilt by amendment applications have in general been unsuccessful (17, 18, 20).

The salutary effect of these N-containing amendments is believed not to be caused by improved suscept resistance, a view given support in work with Ophiobolus (2, 22). In this connection Roberts (19) demonstrated that infection of tomato by Verticillium and subsequent symptom expression are actually favored by nitrogen. Donandt (3) showed the same in experiments with potato and Keyworth and Hewitt (10) with hop. The salutary effect of the barley straw cannot be ascribed to nitrogen.

Under central coastal conditions of California, *V. albo-atrum* forms microsclerotia in the dead tissues of infected tomato plants in the field, mainly between November and March. During the early part of this period, characterized mainly by cool temperatures and low soil moisture, both of which curtail activity of the soil microflora, sclerotial production appears to proceed without check. Since low temperatures definitely favor the production of microsclerotia by Verticillium (25), it is believed that Verticillium would be most vulnerable to depression by competing soil organisms during periods of high soil temperatures.

The reliability of the infection index in measuring inoculum potential is established further by the experiments with mixtures of Verticillium-infested soil and fumigated soil in different proportions. Although the amount of Verticillium is not measured in terms of a unit of the fungus as a spore or sclerotium, the results obtained follow somewhat the trends obtained by Heald (5) in studies of the relation of spore load (inoculum potential) to percentage of stinking smut in wheat, by Glynne (4) of the number of sporangia of *Synchytrium endobioticum* per gm. of soil (inoculum potential) as related to percentage of infected potato plants, and by Leach and

Davey (12) of the number of viable sclerotia of *Sclerotium rolfsii* per sq. ft. of soil to the percentage infection on sugar beets.

SUMMARY

The effect on the inoculum potential of the Verticillium wilt fungus produced by adding various amendments to field-infested soil in pot tests was evaluated by determining the tomato infection index in amended and non-amended portions of the same soil.

When applied in quantities giving approximately equivalent nitrogen levels (0.1–0.13 per cent of the dry weight of the soil), blood meal, fish meal, cottonseed meal, and ammonium sulfate caused substantial reductions in the inoculum potential. One-tenth per cent and 0.01 per cent of Fermate and of Dithane Z–78 caused considerable reduction, whereas similar amounts of Spergon were ineffective.

With certain materials, a decomposition period of 8 weeks gave a greater reduction in inoculum potential than did shorter periods.

Additional evidence in support of the reliability of the infection index method of measuring inoculum potential was obtained by determining the infection indices of mixtures of infested field soil and of chloropicrin-fumigated soil in various proportions.

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INHERITANCE OF RESISTANCE TO SPOT BLOTCH IN BARLEY SEEDLINGS[1]

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(Accepted for publication February 24, 1951)

In 1943 and 1944 spot blotch, caused by *Helminthosporium sativum* Pam., King, and Bakke, reduced barley yields considerably in the North Central states. The disease had not been epidemic for many years; and the variety most widely grown, Wisconsin Barbless, was highly susceptible. This outbreak of the disease renewed the interest in breeding for resistance, and in the mode of inheritance of resistance.

There have been previous reports on the inheritance of mature plant reaction to this disease. In a cross between resistant and susceptible varieties, Hayes et al. (3) found that the reactions were inherited characters, although dependent on more than a single genetic factor, and that resistance was somewhat associated with white color of spike and with rough awns. Griffee (2), on the basis of correlations, concluded that there were at least three genetic factors responsible for mature plant reaction to *H. sativum*, one linked with the factor for 2-rowed vs. 6-rowed, one with the factor for rough vs. smooth awns, and one with the factor for black vs. white spikes.

This paper is a report of efforts to determine more definitely the inheritance and associations of spot-blotch reaction in the seedling stage.

METHODS

Barley seedlings in the second-leaf stage were sprayed thoroughly with a heavy suspension of conidia in water. *H. sativum*[2] was grown on a sterilized medium of equal parts of water and wheat kernels until conidia were produced; the spores were washed off with distilled water, and the suspension was strained through one thickness of cheesecloth. Suspensions were gray or black with conidia. The plants were sprayed with an atomizer on an air pressure line, then placed for 48 hr. in a moist chamber in which the humidity was at or near the saturation point and the temperature between 20° and 25° C. Readings were made within 2 or 3 days. Susceptible plants developed severe leaf necrosis and usually browning of the coleoptile and leaf sheaths at the ground line. Resistant plants had no necrosis or only slight flecking of leaves, and remained white at the ground line. Figure 1 shows the reactions of resistant and susceptible lines.

A few random lots of F_1 and F_2 plants were tested, but most of the work was done with F_3 and F_2 backcross lines in order to distinguish F_2 heterozygotes and dominants. Seed was planted in flats, 10 kernels per line or

[1] Published with the approval of the Director, Wisconsin Agricultural Experiment Station. Acknowledgment is made to Drs. H. L. Shands and J. G. Dickson for counsel during the investigation and the preparation of the manuscript. The photograph was made by Eugene Herrling.

[2] Cultures used were isolated from Wisconsin Barbless kernels obtained from St. Paul, Minnesota; from Fargo, North Dakota; and from several points in Wisconsin.

FIG. 1. Reactions of resistant (left) and susceptible (right) lines of barley to inoculation with *Helminthosporium sativum*.

selection, 18 lines per flat, with parental checks once in each flat. Lines were classified as susceptible, segregating, or resistant, as there was generally a clear-cut response. In segregating lines the numbers of susceptible

and resistant plants were recorded.

In this work seedling plants were tested because of the ease of testing large numbers of plants and lines. Observations and limited field tests have shown agreement between seedling reaction and mature plant reaction.

RESULTS

Inheritance of Resistance

Spot-blotch reactions were determined for nine parental varieties. Two selections of Lion (28 and 36) C.I.923 Sel.[3] are susceptible and are essentially alike in other characters. Lion had been susceptible in earlier work (3). Wisconsin Barbless C.I.5105 also is susceptible and probably derives its reaction from the Lion parent. The seven other parental varieties are resistant: Oderbrucker C.I.4666, Peatland C.I.5267, Colsess IV C.I.5979, Jet C.I.967, O.A.C. 21 C.I.1470, Persicum C.I.6531, and Brachytic C.I.6572.

Certain other varieties, although not used in crosses, were tested for spot-blotch reaction because Hayes et al. (3) had worked with them earlier. The identity of some of these varieties is not positive, since they were referred to by name only. Five varieties that had previously been reported (3) susceptible were resistant in our tests: Trebi C.I.936, Mariout C.I.3577, Gatami C.I.1413, Himalaya C.I.620, and Arequipa C.I.1256. The variety Velvet C.I.4252 was susceptible in the present work although it had been resistant in the earlier work (3). Velvet does have Lion in its parentage.

F_1 seedlings of the following crosses were inoculated: Lion 36 × Oderbrucker, Lion 28 × O.A.C. 21, Lion 28 × Colsess, and Colsess × Lion 36. All seedlings were susceptible, suggesting that the susceptibility of Lion is dominant.

The inoculated F_2 plants of Lion 28 × Oderbrucker gave 116 susceptible and 28 resistant plants, which is a satisfactory fit with the ratio expected if inheritance of spot-blotch susceptibility is due to a single dominant gene ($\chi^2 = 2.08$, 1 d.f., P lies between 0.20 and 0.10). F_2 plants of Lion 28 × Colsess IV (homozygous green) gave 161 susceptible and 75 resistant plants. This total was composed of families from nine different F_1 plants. The fit with a 3 : 1 ratio is again satisfactory ($\chi^2 = 16.85$, 9 d.f., P lies between 0.10 and 0.05). The cross Lion 28 × Colsess IV (heterozygous xantha) gave 69 susceptible and 19 resistant F_2 plants. Families from five F_1 plants were involved. The fit with a 3 : 1 ratio is satisfactory ($\chi^2 = 8.43$, 5 d.f., P lies between 0.20 and 0.10).

In order to distinguish F_2 homozygotes and heterozygotes the reactions of F_3 and backcross lines were studied. The distribution of F_3 and backcross lines for several crosses is in table 1. In every case involving a resistant and a susceptible parent the evidence points to a single factor pair difference. Using only 10 plants per line there was difficulty in distinguish-

[3] C.I. refers to the accession number of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

ing the segregating and susceptible lines in crosses involving Colsess IV (heterozygous xantha), since usually the xantha plants were dead before their disease reaction could be obtained. The resistant classes could be determined accurately because resistance is recessive. If the susceptible and segregating classes of these crosses involving xantha are combined, the fit with a 3 : 1 ratio is satisfactory. In the two crosses involving only resistant parents, only resistant F_3 lines were obtained, suggesting that these parents have at least one resistance factor in common.

In certain lines classified as segregating, the numbers of resistant and

TABLE 1.—*Distribution of F_3 lines of several crosses for spot-blotch reaction*

Cross	Number of F_3 lines in class:			Theoretical ratio	Chi-square	P
	Susceptible	Segregating	Resistant			
Lion 28 × Oderbrucker	49	92	43	1 : 2 : 1	0.39	0.95–0.50
Lion 36 × Oderbrucker	44	70	44	1 : 2 : 1	2.05	0.50–0.30
Oderbrucker × F_1 (Lion 28 × Oderbrucker)	0	69	90	1 : 1	2.52	0.20–0.10
Lion 28 × F_1 (Lion 28 × Oderbrucker)	54	48	0	1 : 1	0.24	0.95–0.50
Wisconsin Barbless × Peatland	36	61	31	1 : 2 : 1	0.67	0.95–0.50
Colsess IV (Green) × Lion 28	28	52	28	1 : 2 : 1	0.15	0.95–0.50
Colsess IV (Het. xantha) × Lion 28	53	60	28	3 : 1[a]	2.01	0.20–0.10
Colsess IV (Het. xantha) × Lion 36	183	239	139	3 : 1[a]	0.01	0.95–0.50
Lion 28 × Colsess IV (Het. xantha)	85	120	67	3 : 1[a]	0.02	0.95–0.50
Persicum × Wisconsin Barbless	68	102	70	1 : 2 : 1	5.44	0.10–0.05
Wisconsin Barbless × Jet	66	120	58	1 : 2 : 1	0.59	0.95–0.50
F_1 (Wis. Barbless × Jet) × Jet	0	27	19	1 : 1	1.39	0.30–0.20
Oderbrucker × Colsess IV	0	0	49	.....	.....	.....
Persicum × Brachytic	0	0	28	.....	.....	.....

[a] Susceptible and segregating lines combined.

susceptible plants per line were counted. The average number of plants for several crosses was as follows: Lion 36 × Oderbrucker, 70 lines, 7.71 susceptible, 2.29 resistant; Oderbrucker × F_1 (Lion 28 × Oderbrucker), 69 lines, 7.54 susceptible, 2.46 resistant; Lion 28 × F_1 (Lion 28 × Oderbrucker), 48 lines, 7.56 susceptible, 2.44 resistant; and Wisconsin Barbless × Peatland, 61 lines, 7.30 susceptible, 2.70 resistant. With 10 plants tested per line, an average of 7.75 plants would be expected to be susceptible and 2.25 resistant. The variation from the expected was not great in any case. This is further evidence that susceptibility to spot blotch is dominant, and that the reaction is conditioned by a single factor pair.

Association of Characters

In Lion 36 × Oderbrucker the F_3 lines were classified for several morphological characters as well as for splot-blotch reaction. Lion 36 has black lemma and pericarp (BB, group II),[4] smooth awns (rr, group V) long-

[4] Symbols and linkage groups from Robertson et al. (4, 5).

TABLE 2.—*Distribution of F_3 lines with respect to spot-blotch reaction and morphological characters*

Cross	Number of lines in each spot-blotch class: Susceptible			Segregating			Resistant			P lies[a] between
Lion 36 × Oderbrucker	BB–6	Bb–18	bb–13	BB–16	Bb–31	bb–16	BB–10	Bb–20	bb–10	0.95–0.50
	RR–9	Rr–18	rr–10	RR–13	Rr–32	rr–18	RR–12	Rr–20	rr–8	0.95–0.50
	SS–8	Ss–23	ss–6	SS–20	Ss–33	ss–10	SS–12	**Ss–16**	ss–12	0.50–0.30
	E_2E_2–9	E_2e_2–18	e_2e_2–10	E_2E_2–16	E_2e_2–31	e_2e_2–16	E_2E_2–3	E_2e_2–28	e_2e_2–9	0.30–0.20
Oderbrucker × F_1 (Lion 28 × Oderbrucker)	.....	.....	.....	.....	Bb–27	bb–32	.....	Bb–40	bb–41	0.30–0.20
	.....	.....	.....	.....	Ss–28	ss–31	.....	Ss–41	ss–40	0.50–0.30
Lion 28 × F_1 (Lion 28 × Oderbrucker)	.....	Rr–23	rr–30	.....	Rr–19	rr–29	.....	.....	.....	0.50–0.30
	.....	E_2e_2–26	e_2e_2–27	.....	E_2e_2–26	e_2e_2–22	.....	.....	.....	0.95–0.50
Wisconsin Barbless × Peatland	RR & Rr–29[b]		rr–7	RR & Rr–49		rr–12	RR & Rr–23		rr–8	0.95–0.50
	TT & Tt–27		tt–9	TT & Tt–44		tt–17	TT & Tt–21		tt–10	0.95–0.50
Persicum × Wisconsin Barbless	VV–18	Vv–32	vv–18	VV–19	Vv–53	vv–30	VV–21	Vv–25	vv–24	0.10–0.05
Wisconsin Barbless × Jet	VV–18	Vv–30	vv–18	VV–27	Vv–61	vv–32	VV–13	Vv–27	vv–18	0.99–0.95
	NN & Nn–49[b]		nn–17	NN & Nn–92		nn–28	NN & Nn–42		nn–16	0.99–0.95
	RR & Rr–58		rr–8	RR & Rr–93		rr–27	RR & Rr–40		rr–18	0.20–0.10
	BB & Bb–54		bb–12	BB & Bb–90		bb–30	BB & Bb–40		bb–18	0.95–0.50
F_1 (Wisconsin Barbless × Jet) × Jet	.....	.....	.....	VV–18	Vv–9	.....	VV–10	Vv–9	.....	0.20–0.10
	.....	.....	.....	.....	Nn–13	nn–14	.....	Nn–7	nn–12	0.50–0.30
Colsess IV (Het. xantha) × Lion 36[c]	BB & Bb–309		bb–112	.....	.....	.....	BB & Bb–104		bb–36	0.95–0.50
	KK & Kk–321		kk–100	.....	.....	.....	KK & Kk–101		kk–39	0.95–0.50
	SS & Ss–313		ss–108	.....	.....	.....	SS & Ss–110		ss–30	0.95–0.50
Lion 28 × Colsess IV[c] (Het. xantha)	BB & Bb–154		bb–51	.....	.....	.....	BB & Bb–49		bb–17	> 0.99
	KK & Kk–150		kk–55	.....	.....	.....	KK & Kk–45		kk–21	0.95–0.50
	SS & Ss–162		ss–43	.....	.....	.....	SS & Ss–51		ss–15	0.95–0.50

[a] Two independent factors assumed.

[b] Lines of these crosses were not grown to maturity for classification of morphological characters.

[c] Spot-blotch tests were made on F_3 lines, but separation of susceptible and segregating lines was not possible. Morphological characters classified in F_3.

haired rachillas (SS, group V), and short glume awns (e_2e_2, group undetermined). On the other hand, Oderbrucker has white lemma and pericarp (bb), rough awns (RR), short-haired rachillas (ss), and long glume awns (E_2E_2). The distribution of the F_3 lines for these characters and for spot-blotch reaction (Table 2) fit satisfactorily the ratios expected on the assumption of two independent factors. In the backcrosses involving these parents, all characters again proved to be independent of spot-blotch reaction.

Wisconsin Barbless is smooth-awned (rr) and stem-rust susceptible (tt, group VII); Peatland is rough-awned (RR) and stem-rust resistant (TT). Persicum is 2-rowed (VV, group I), and Wisconsin Barbless 6-rowed (vv). Wisconsin Barbless is 6-rowed (vv), hulled (NN, group III), smooth (rr), and white (bb). Jet is 2-rowed (VV), naked (nn), rough (RR), and black (BB). These characters all appear to be independent of spot-blotch reaction, judging from the distribution of F_3 and backcross lines (Table 2).

Several characters were also studied in the cross Colsess IV × Lion for possible linkage relationships with the spot-blotch factor. Colsess IV (heterozygous xantha) is hooded (KK, group IV), has white lemma and pericarp (bb), short-haired rachillas (ss), and is heterozygous for the chlorophyll deficiency xantha (Xcxc, group VI). Lion 28 and Lion 36 are awned (kk), have black lemma and pericarp (BB), and long-haired rachillas (SS). The distributions for two crosses of these varieties are in table 2. The crosses are essentially reciprocals. For these crosses, as in table 1, the lines susceptible and segregating for spot-blotch reaction are combined. In all cases there is good fit with the 9:3:3:1 ratio expected on the basis of two independent factors. No direct test with the xantha character was obtained, but since the loss of homozygous xantha plants in the F_2 did not upset the spot-blotch segregation in the F_2 or F_3, it may be assumed that the two characters are independent.

The relation of spot-blotch reaction to stripe[5] reaction was studied in Lion × Oderbrucker and the backcrosses to both parents. These two diseases are of very different nature, as spot blotch results from local infection and stripe from systemic infection. The stripe reaction of this material has been previously reported (1). The same lines were tested for their reaction to spot blotch. The simultaneous distribution of F_3 lines for the two diseases is in table 3. Since in Lion × Oderbrucker the average stripe infection was 21, 31, and 42 per cent for lines susceptible, segregating, and resistant to spot blotch, respectively, an association of splot-blotch susceptibility with stripe resistance is suggested. This is borne out by a high Chi-square value in a test for independence. Association is also apparent in Oderbrucker × F_1 (Lion 28 × Oderbrucker), as the spot-blotch resistant lines had a higher average stripe infection (67 per cent) than the lines segregating for spot-blotch reaction (51 per cent). The Chi-square test indicates an association of the two characters. However, in Lion 28 × F_1

[5] Caused by *Helminthosporium gramineum* Rabh.

(Lion 28 × Oderbrucker) the association with spot-blotch reaction is not evident, perhaps because all lines were either resistant or moderately resistant to stripe as would be expected in a backcross to the resistant parent when stripe resistance is at least partially dominant.

Marker characters in all seven linkage groups have been observed for possible associations with spot-blotch reaction and none was found. The

TABLE 3.—*Distribution of F_3 and back-cross lines for reaction to stripe (culture C-1) and spot blotch*

Cross	Spot-blotch class[a]	Number of lines in stripe classes:[b] 5	15	25	35	45	55	65	75	85	Total number of lines	Av. percentage stripe
Lion 36 × Oderbrucker	S	12	9	6	6	2	1	1	0	0	37	21
	Seg	3	15	13	15	9	5	2	1	0	63	31
	R	2	2	9	6	6	8	3	4	0	40	42
Test for independence χ^2—39.24, $P < 0.01$												
Oderbrucker × F_1 (Lion 28 × Oderbrucker)[c]	Seg	0	1	6	7	12	18	9	5	1	59	51
	R	0	0	0	1	5	17	23	22	13	81	67
Test for independence χ^2—38.85, $P < 0.01$												
Lion × F_1 (Lion 28 × Oderbrucker)	S	7	24	15	4	3	0	0	0	0	53	20
	Seg	2	24	14	3	3	0	0	1	0	47	21
Test for independence χ^2—2.75, P lies between 0.95–0.50												

[a] S = susceptible to spot blotch; Seg = segregating; R = resistant.

[b] Because some groups were small, combinations were made for the Chi-square tests as follows:

Lion × Oderbrucker—segregating for spot blotch, 65 and 75 per cent classes; resistant to spot blotch, 65 and 75 per cent classes.

Backcross to Oderbrucker—segregating for spot blotch, 15, 25, and 35 per cent classes; resistant to spot blotch, 85 and 95 per cent classes.

Backcross to Lion 28—segregating for spot blotch, 1 line from 75 per cent class omitted.

[c] Four lines classed as spot-blotch susceptible omitted.

gene which governs spot-blotch reaction may be located in one of the groups, but too far from the marker gene for any association to be readily detected with the small populations used. Further work is needed to clarify this point.

DISCUSSION

The results of the studies reported in this paper do not agree with those obtained earlier by Hayes et al. (3) and Griffee (2), as they reported different reactions for certain varieties, more than one genetic factor in the inheritance of reactions, and associations with certain morphological characters. A part of this discrepancy may be due to differences in the fungus strains involved, since their work was done at a different place and more than 20 years ago. Another difference may be in the methods of inoculation and the age of the plants at the time of inoculation. All tests reported here were made on seedlings in the greenhouse during the winter months and inoculations made by spraying seedlings with spore suspensions. In the earlier work field tests were made on infested soil, plants were sprayed

with inoculum several times, and readings were made as plants reached maturity; greenhouse tests were made by inoculating the soil before the seed was planted. In the present work the difference in plant reaction seemed sharp enough that only ratings of resistant or susceptible were used, while in the earlier report infection on various parts of the plant was noted as heavy, medium, light, or trace. These differences may partially explain the differences in results and conclusions.

SUMMARY

The difference between susceptibility and resistance to spot blotch in barley seedlings appeared to be due to a single factor pair, with susceptibility dominant. This susceptibility has been found only in Lion and some of its derivatives.

There was no detectable association of spot-blotch reaction with any of the seven linkage groups represented by 2-rowed vs. 6-rowed, lemma and pericarp color, hulled vs. naked, hoods vs. awns, barbing of awns and rachilla hair length, the xantha chlorophyll deficiency, or reaction to stem rust. In one cross spot-blotch susceptibility appeared to be associated with stripe resistance.

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THE INSECT VECTOR RELATIONSHIPS OF THE FILAREE RED-LEAF VIRUS, WITH SPECIAL REFERENCE TO A LATENT-PERIOD DIFFERENCE BETWEEN NYMPHS AND ADULTS IN MACROSIPHUM GERANICOLA (LAMBERS)[1]

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(Accepted for publication February 28, 1951)

The filaree red-leaf virus disease, the transmission of the virus by several aphid species, and its persistence in *Macrosiphum geranicola* were recently described by Frazier (3). The virus belongs to a group of about 15 viruses (e.g., potato leaf roll, sugar-beet yellows, etc.) known to persist in their aphid vectors. This paper presents the results of a study of the vector relationships of the red-leaf virus in the body of its chief aphid vector, *M. geranicola*.

MATERIALS AND METHODS

Aphid species[3] other than *M. geranicola* were used only in the section entitled "Vectors." Noninfective aphid colonies of each species used in these tests were reared from single aphids. The aphid colonies were maintained on healthy plants of white-stem (*Erodium moschatum*) or red-stem (*E. cicutarium*) filaree in the greenhouse. Healthy seedlings of white-stem or in some cases red-stem filaree, growing in 3- or 4-in. pots, were used as test plants.

All seedlings were fumigated with nicotine for 10 min. or longer when the caged aphids were removed from them. Test plants were sprayed about once a week with parathion, and living aphids were only rarely noted on test plants in the greenhouse. Inoculum plants were sprayed as regularly with either nicotine sulfate or tetraethyl pyrophosphate. Test plants were observed for at least 30 days unless symptoms developed before that time.

Individual aphids were transferred to test plants by means of a moistened camel's-hair brush.

Adequate controls were used in every experiment with one exception (noted in the text), and all control plants remained healthy.

[1] Condensed portion of a thesis presented to the graduate division of the University of California, June 1950, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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[3] The writer is indebted to Professor M. A. Palmer for the determination of *Macrosiphum geranicola* (Lambers), *M. pelargonii* (Kaltenbach), and *Myzus solani* (Kaltenbach), and to Professor E. O. Essig who determined all other species of aphids used during this investigation.

Macrosiphum geranicola was collected from *Erodium* sp. and is believed to be identical with the form used by Frazier (3). A more recent communication from Palmer indicates that *M. geranicola* is more properly designated *M. zerozalphum* Knowlton, but continues to uphold the distinction between this aphid and *M. pelargonii*.

DEFINITIONS

The definitions of the terms used in this paper follow Sylvester (7) as closely as possible, and the terms themselves are largely self-explanatory. Certain exceptions to Sylvester's definitions are noted below:

Acquisition feeding and inoculation feeding replace "infection feeding" and "test feeding," respectively.

Sylvester correctly eliminated the prepenetration time from the measurement of the acquisition and inoculation thresholds. For practical reasons it was impossible to eliminate the prepenetration time in measuring the long acquisition threshold of the red-leaf virus.

ACQUISITION THRESHOLD

Because in early tests *M. geranicola* failed to acquire the red-leaf virus in about 2 hr., young nymphs were transferred to diseased white-stem filaree plants and allowed to feed on them for either 2 or 3 hr. $\pm$ 10½ min. The nymphs were then transferred to healthy test seedlings for a 6-day inoculation feeding. Seven of 37 nymphs infected their test plants following the 3-hr. acquisition feeding. None of 48 nymphs proved infective after a 2-hr. acquisition feeding, although the availability of the virus in the diseased plants was proved. Two hours are sufficient for nymphs to complete relatively long continuous penetrations. Thus the acquisition threshold lies between 2 and 3 hr.

INOCULATION THRESHOLD

The minimum feeding time needed by infective vectors to inoculate healthy plants was determined as follows: Adults from colonies maintained 6 days or longer on inoculum were used exclusively. White-stem filaree was used for inoculum and test plants. Inoculation feedings were timed with a stop watch. Using one aphid per test plant, five replications of seven test plants each were run for each time interval.

Three of 35 plants became diseased following 15-min. inoculation feedings. All 35 test plants remained healthy after 10-min. inoculation feedings, although the infectivity of the aphid colonies was substantiated. Thus the inoculation threshold lies between 10 and 15 min.

AVAILABILITY THRESHOLD

White-stem filaree seedlings were inoculated by allowing five large aphids from diseased plants to feed on each for 5 or 6 hr. The aphids were then removed and the inoculated plants fumigated and placed in the greenhouse. Early in the second day after inoculation, small noninfective nymphs were placed on the inoculated plants. Five nymphs fed for 18–24 hr. on the inoculated plants during the day on which they were moved to test plants. The nymphs fed for 6 days on the test plants.

Although 135 nymphs survived the 6-day inoculation feedings, they failed to recover virus from 30 successfully infected plants during the sec-

ond day after inoculation. Virus was recovered from 9 of 37 plants during the third day, from 7 of 8 during the fourth, and from 10 of 10 during the fifth. Thus the red-leaf virus becomes available to its vector during the third day after inoculation of a previously healthy plant.

EFFECTS OF FASTING ON VIRUS TRANSMISSION

Watson (8) and Watson and Roberts (11) were instrumental in demonstrating that fasting before a short acquisition feeding increases the efficiency of an aphid vector in transmitting a nonpersistent virus. An enzyme inactivator produced during feeding was thought to be responsible for this phenomenon.

Watson (10) also showed that fasting before the acquisition feeding has no effect on transmission of the persistent aphid-borne sugar-beet yellows virus. She felt that vector response to preliminary fasting separated persistent from nonpersistent viruses better than did the length of the retention period.

Although the long acquisition threshold of the red-leaf virus makes it impossible to test the effect of fasting on virus acquisition during short feedings, the effect of fasting before relatively long acquisition feedings was tested as follows: Similar leaves from a single diseased white-stem filaree plant were divided into two similar groups and placed in vials of water. Adult apterae, previously fasted for 12–19 hr., were placed on one group, and similar but unfasted adult apterae were placed on the other. Aphids of each group fed on the diseased leaves for 6 hr. $\pm$ 14 min. When the acquisition feedings ended, the aphids were moved to healthy test plants, on which they fed for at least 6 days.

Three of 56 fasted aphids infected their test plants; five of 55 unfasted aphids infected theirs. Thus fasting prior to the acquisition feeding did not increase vector efficiency.

Other investigators (2, 10) report that fasting after the acquisition feeding unexpectedly led to a temporary or permanent decrease in the ability of the vector of a persistent virus to transmit its virus. To test this possibility with the red-leaf virus, aphids were placed on a diseased white-stem filaree plant and fed thereon for at least 6 days. Adult apterae were then removed from inoculum and, after fasting 20 to 24 hr., were placed on healthy white-stem filaree test plants for 1 hr. An equal number of unfasted apterae from the same diseased plant were given similar 1-hr. inoculation feedings. This experiment was repeated using inoculation feedings of 15 instead of 60 min. The 15-min. feedings were intervals of continuous penetration timed with a stop watch. The 60-min. feedings began with continuous penetration for 5 min., but the aphid was not observed again until the end of the period. When allowed 60-min. inoculation feedings, 13 of 34 unfasted aphids infected the test plants, and 10 of 33 fasted aphids did so. Given 15-min. inoculation feedings, three of 35 unfasted aphids and four of 35 fasted ones infected the test plants. Thus fasting following the acquisi-

tion feeding has no definite effect on the transmission of the red-leaf virus by its vector. In this respect the transmission of the red-leaf virus differs from that of sugar-beet yellows virus (10), although both are persistent aphid-borne viruses.

LATENT PERIOD

Preliminary work indicated that a latent period may be required by *M. geranicola* in the transmission of the red-leaf virus. Therefore, groups of 10 small noninfective nymphs were placed on diseased plants for approximately 6 hr. Each group was then serially transferred to healthy test plants. The periods for which the groups remained on successive test plants were as follows: first healthy plant, 12 hr.; second, 6 hr.; third, 6 hr.; fourth, 18 hr.; fifth, 4 to 8 days. Red-stem filaree was used in this experiment, the only one in which controls were not used.

One hundred twenty-six nymphs of 14 aphid groups reached the fifth test plant (Table 1). The results indicate that the latent period was at

TABLE 1.—*Latent period of the filaree red-leaf virus in young nymphs of Macrosiphum geranicola*

Aphid groups[a]	Feeding time and infection[b] on five successive test plants				
	1 12 hr.	2 6 hr.	3 6 hr.	4 18 hr.	5 4–8 days
1	+	−	+	+	+
2–5	−	−	+	+	+
6–13	−	−	−	+	+
14	−	−	−	−	+

[a] Each of the 14 groups contained 10 nymphs that were transferred to the test plants after a 6-hr. acquisition feeding.

[b] + indicates plant became diseased; − indicates plant remained healthy.

least 18 hr. for four groups, at least 24 hr. for eight groups, and at least 42 hr. for one group. The first group infected the first test plant; thus a latent period was not determinable, but it could not have exceeded 15 hr. because of the 3-hr. acquisition threshold. Once a group's latent period was over, every succeeding test plant was infected (except that the first group infected the first and third test plants but not the second). Since there was also other evidence that the early infection caused by the first group may have resulted from contamination, most, and possibly all, nymphs had a minimum latent period of 18–27 hr. or more. The total time spent on the first two test plants without infecting them (except in the doubtful case of the first group) is 18 hr., and 27 hr. is the time spent on inoculum and the first three minus the 3-hr. acquisition threshold.

During daily serial transfers following 24-hr. acquisition feedings in preliminary work, 35 per cent of all infective nymphs caused infections within 48 hr. of the time they were placed on diseased plants, and 76 per cent did so within 72 hr. On the other hand, no infective adult caused

infection within 48 hr. of the time it was placed on a diseased plant, and only 43 per cent of them infected plants within 72 hr. The latent period difference thus indicated was tested further as follows: White-stem filaree was used for maintaining all aphid colonies and for all inoculum and test plants. Adults from one noninfective colony were placed on healthy plant A. After 12 hr. the adults were removed, leaving their descendants to be reared; $4\frac{1}{2}$ days later, a second group of adults from the same noninfective colony was placed on healthy plant B for 12 hr. After this period, aphids were moved to inoculum as follows: Nymphs from plant B, listed in table 2

TABLE 2.—*Latent period of the filaree red-leaf virus in infective nymphs of two ages, and adults, of Macrosiphum geranicola; tested individually in seven serial transfers following a 6-hr. acquisition feeding*

Insect No.	Transmission results[a]																								
	Nymphs ¼ day old[b]							Nymphs 5¼ days old[b]							Adults										
	Test plant series																								
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7				
	Feeding time (hours) on successive test plants																								
	18	12	12	12	12	24	72	18	12	12	12	12	24	72	18	12	12	12	12	24	72				
1	−	+	+	+	+	+	+	−	−	−	+	−	−	+	−	−	−	−	+	+	+				
2	−	−	+	−	+	+	+	−	−	−	+	−	+	+	−	−	−	−	−	+	+				
3	−	−	+	−	+	+	+	−	−	−	−	+	+	+	−	−	−	−	−	+	+				
4	−	−	+	+	+	+	+	−	−	−	−	−	+	+	−	−	−	−	−	−	+				
5	−	−	+	−	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	+				
6	−	−	+	−	+	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−	+				
7	−	−	+	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−	+				
8	−	−	−	+	+	−	+	−	−	−	−	−	−	+	−	−	−	0	−	−	+				
9	−	−	−	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	+				
10	−	−	−	+	−	+	+								−	−	−	−	−	−	+				
11	−	−	−	+	+	−	+																		
12	−	−	−	+	−	+	+																		
13	−	−	−	+	−	+	+																		
14	−	−	−	+	+	+	+																		
15	0	−	−	+	+	+	+																		
16	−	−	−	−	+	+	+																		
17	−	−	−	−	+	+	+																		
18	−	−	−	−	−	+	+																		
19	−	−	−	−	−	+	0																		
20	−	−	−	−	−	+	−																		
21	−	−	−	−	−	−	+																		

[a] + indicates plant became diseased; − indicates plant remained healthy; 0 indicates plant dead or unreadable.

[b] Average age at the time the aphids were placed on inoculum.

under their probable average age of ¼ day, were first moved to diseased plant C; adults from plant B were moved to second diseased plant D; and the $5\frac{1}{4}$-day-old nymphs from plant A were placed on plant D. At the end of the acquisition feeding, each of five nymphs from each of the three age groups was moved to its first healthy test plant. Each aphid then fed successively on seven test plants. The aphids remained on the plants for the following periods: diseased plants, 6 hr.; first test plant, 18 hr.; second,

third, fourth, and fifth, 12 hr. each; sixth, 24 hr.; seventh, 72 hr. The time needed for each successive transfer of 15 test aphids never exceeded 30 min. As controls, two 5¼-day-old nymphs from plant A and three adults from plant B were each transferred to a set of seven successive test plants without spending any time on diseased plant C or D.

Thirty-three per cent of the infective ¼-day-old nymphs infected their third test plant, and 81 per cent infected one or more of the first five test plants (Table 2). In contrast, no infective adult infected any of the first four test plants and only 10 per cent infected the fifth. The minimum latent period of ¼-day-old nymphs lies between 18 and 33 hr. (table 1 indicated 18–27 hr. for young nymphs). The minimum for adults is between 54 and 69 hr. Therefore, the minimum and average latent periods of the red-leaf virus are shorter in young nymphs than in adults. Osborn's tables (6) indicate that the same phenomenon may occur in the transmission of pea enation mosaic by *Macrosiphum pisi.*

In experiments rather similar to the above, to determine the effect of the length of the acquisition feeding on the length of the latent period in adults of *M. geranicola,* only three, nine, and 12 adults became infective following acquisition feedings of 6, 12, and 24 hr., respectively. About 25 adults were tested for each of the three acquisition feeding periods used, and each adult was tested individually. The low number of adults becoming infective after the 6-hr. acquisition feeding period makes it impossible to draw definite conclusions regarding the effect of the length of the acquisition feeding period on the length of the latent period from this experiment. However, if one 1) combines the results from the ten infective adults of table 2 with those of the three adults which became infective after a 6-hr. acquisition feeding period in this test, and 2) compares these combined results with those of the infective adults given 12- and 24-hr. acquisition feedings in this test, one finds that lengthening the acquisition feeding probably has no effect on the length of the minimum latent period but may tend to shorten the average latent period slightly. In the 6-, 12-, and 24-hr. acquisition feeding tests reported here, as in those of the 10 infective adults of table 2, and of the preliminary tests previously referred to, no infective adult infected any test plant within 48 hr. of the start of the acquisition feeding, although one adult did infect its first plant within 48–60 hr. of that time. This provides additional evidence that adults have longer latent periods than do young nymphs.

INFLUENCE OF VECTOR AGE ON ABILITY TO ACQUIRE THE RED-LEAF VIRUS

No definitive tests were made on this subject, but results obtained during the latent-period studies suggested that young nymphs may acquire the virus more readily than adults. In preliminary nymph-adult latent-period studies, 20 of 23 young nymphs (average age, ½ day when placed on inoculum) and 14 to 20 adults acquired the virus during 1-day acquisition feeding periods. In later studies (Table 2) 21 of 28 nymphs ¼-day-old and 10

of 30 adults acquired the virus in 6-hr. acquisition feedings. Adding these results, 41 of 51 young nymphs and 24 of 50 adults acquired the virus. In the preliminary studies referred to above, noninfective aphids were not counted unless they survived for 6 days after being placed on inoculum; noninfective aphids from table 2 were required to survive for 7 days. These survival requirements were adequate to bring out the latent-period differences between nymphs and adults, and it was evident that all or almost all the infective nymphs were discovered by them. It is more likely that some of the adults acquired the virus but were not given sufficient time to complete their latent periods. Thus, the above results may have exaggerated the difference between nymphs and adults in the proportion of aphids becoming infective following a given acquisition feeding period. Nevertheless, the writer feels that such differences were certainly present.

VECTORS

Frazier (3) found that *Macrosiphum geranicola, M. solanifolii,* and *Myzus solani* are vectors of the red-leaf virus, but he failed to obtain transmission with *Myzus persicae, M. ornatus,* and *Capitophorus fragaefolii.* During the present investigations white-stem filaree was used for all inocu-

TABLE 3.—*Tests of the ability of eight aphid species to act as vectors of the filaree red-leaf virus*

Aphid species tested	Number of aphids used per test plant	Number of plants	
		Inoculated	Infected
Macrosiphum geranicola (Lambers)	1	10	9
M. pelargonii (Kaltenbach)	1	20	8
M. solanifolii (Ashmead)	1	25	4
Myzus solani (Kaltenbach)	1	30	10
M. persicae (Sulzer)	10	34	0
Rhopalosiphum prunifoliae Fitch	1	17	1
	10	15	12
Aphis rumicis Linnaeus	10	30	18
A. gossypii Glover	10	30	0

Rhopalosiphum prunifoliae to the list of vectors, and *Aphis gossypii* to the nonvectors, reported by Frazier. Tests with the other species confirm the conclusions reached by Frazier, but higher percentages of transmission (for equal numbers of aphids per test plant) were generally obtained in the lum and test plants in testing the ability of certain aphid species to act as vectors of the virus. Aphids were confined on inoculum for at least 4 days and on test plants for at least 2 days. Only adults were transferred to the test plants in most cases, but large nymphs were also used in tests of *Macrosiphum pelargonii, Aphis gossypii,* and *Rhopalosiphum prunifoliae.* Three to six diseased plants were used as inoculum for each aphid species except *Macrosiphum geranicola;* one control plant (without aphids) was used for each test plant.

The results (Table 3) add *Macrosiphum pelargonii, Aphis rumicis,* and

present work. This may have resulted from the use of longer acquisition and inoculation feedings in the present work.

The plant species used for inoculum and test plants may also influence the amount of transmission obtained, since both *Rhopalosiphum prunifoliae* and *Aphis medicaginis* were very poor vectors when red-stem filaree was used, but relatively good ones when white-stem was used. The tests with *A. medicaginis* were never completed, however, and its vector ability remains unknown.

DISCUSSION

The writer attempted to give adequate consideration, in each step of the present work, to the influence of the facts discovered in other steps. For example, the use of long acquisition feedings and of adults should have insured accurate results in the inoculation threshold tests.

In general, the results obtained in the present study are comparable to those obtained by other workers with other persistent aphid-borne viruses. The relatively long acquisition threshold and the comparative ease with which young nymphs acquire virus deserve some discussion, however.

The relatively long acquisition threshold of the red-leaf virus may result from 1) low virus concentration in the plant, 2) failure of the nymphs to reach virus-containing tissue quickly, or 3) a long latent period caused by the short acquisition feeding. Since adults reach tissue suitable for inoculation in 15 min., whereas no evidence was discovered that lengthening the acquisition feeding from 6 to 24 hr. greatly affected the length of the latent period, it is perhaps most likely that the nymphs did reach virus-containing tissue (probably phloem) but failed to acquire demonstrable amounts of virus because of low virus concentration in the plant. Yet the fact that the virus multiplies rapidly enough to be recoverable the third day after inoculation and to produce symptoms on the fifth day (3) makes this reasoning appear anomalous. Rapid virus multiplication to a low maximum concentration in the plant or a decrease in concentration following an early maximum might explain this anomaly.

The ability of the beet leafhopper to acquire the curly-top virus gradually decreases as the adults age (4), whereas the spotted-wilt vectors can acquire virus as nymphs but not as adults (1, 5). The shorter latent period in young nymphs of *Macrosiphum geranicola*, the termination of the experiments in 6 or 7 days, and other factors may have increased the discrepancy between nymphs and adults in the acquisition of the red-leaf virus. Nevertheless, it is likely that nymphs do acquire the virus more readily than adults. In this respect, the situation regarding the red-leaf virus is intermediate between that of curly top and that of spotted wilt cited above. The reason for the more ready acquisition of virus by nymphs is unknown, but physiological changes similar to those cited by Freitag (4) furnish the most likely explanation.

The status of the latent periods observed in vectors of certain plant

viruses is unsettled. Watson (9, 10) believes that latent periods, as absolute time minima needed for the development of infectivity by the vector, do not exist. Other workers feel that latent periods in the above sense do occur, and various theories have been offered to explain them. The two most likely of these are: 1) latent periods represent the time required for virus to reach the salivary glands via the stomach, gut wall, and blood; and 2) latent periods represent time needed for virus multiplication within the vector to reach the point enabling the vector to infect healthy plants.

Watson explains the observed latent periods as follows: 1) The short acquisition feedings used in latent-period tests produce conditions of weak vector infectivity during which the chances of obtaining early infections are slight, and 2) there is no evidence of a lower time limit for feeding periods, separately or in combination, below which sufficient trials will not demonstrate infectivity.

The results of this investigation indicate that some factor other than those cited by Watson is responsible for the observed latent period of the red-leaf virus because: 1) Increasing the acquisition feeding from 6 to 24 hr. had little or no effect on the average latent period and none on the minimum time required for virus transmission. 2) After a latent period of 18 to 33 hr., 15 of 21 infective young nymphs became actively infective during the following 36 hr. Twelve of 14 nymph groups became demonstrably infective within 24 hr. following a minimum latent period of 18 to 27 hr. The 6-hr. acquisition feedings used in these tests were twice the length of the acquisition threshold, and the individually tested nymphs produced repeated infections. If weakly infective vectors and chance were responsible for the latent period, the demonstrated latent periods probably would not have fallen into such a well-defined time interval. 3) The difference in the length of the latent period between nymphs and adults indicates that some factor other than those cited by Watson is involved in determining the latent period of the red-leaf virus.

Therefore, although it must be admitted that occasional examples of early transmission may occur (as may have happened in one doubtful instance reported in this paper), some factor other than the low chances of obtaining early infection following short acquisition feedings is responsible for the normal latent periods in nymphs and adults of *M. geranicola*. Whether this factor is time needed for virus multiplication in the vector, time needed for virus passage through the vector, or some other matter is not known.

SUMMARY

Macrosiphum geranicola requires between 2 and 3 hr. to acquire the red-leaf virus from a diseased plant but only between 10 and 15 min. to infect a healthy one. The red-leaf virus becomes available to *M. geranicola* during the third day after the infection of a healthy filaree plant. Forcing infective individuals of *M. geranicola* to fast for long periods after acquiring

the virus does not affect their infectivity, nor does fasting before the acquisition feeding cause aphids to acquire greater infectivity or to become infective faster.

The red-leaf virus undergoes a minimum latent period of between 18 and 27 hr. in young nymphs of *M. geranicola*. The minimum and average latent periods in adults are even longer, and no adult succeeded in infecting a healthy plant within 48 hr. of the time it was placed on inoculum. The length of the acquisition feeding probably has little or no effect on the length of the minimum latent period but may shorten the average latent period slightly. The cause of the latent period was discussed.

Macrosiphum pelargonii, *Aphis rumicis*, and *Rhopalosiphum prunifoliae* are additional vectors of the red-leaf virus; *Myzus persicae* and *Aphis gossypii* failed to transmit it.

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CONTROL OF DECAY IN PREPACKAGED SPINACH[1]

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(Accepted for publication March 12, 1951)

A large proportion of fresh spinach now being sold at retail is trimmed, washed, and packaged in bags of transparent films. Prepackaged spinach has a relatively short marketing period because of the rapid development of bacterial soft rot within the bags. Means for preventing or reducing decay were studied during 1948 and 1949 and a preliminary report has been released to the trade (2). The present paper constitutes a report on four phases of the study, namely, the use of chlorine compounds in wash water, the use of silver-treated Cellophane, a comparison of different temperatures for holding spinach after packaging, and the effect of centrifugal drying on washed spinach.

CHLORINE COMPOUNDS IN WASH WATER

High concentrations of sodium hypochlorite solution have reportedly reduced the number of bacteria on spinach leaves, but have not controlled decay of prepackaged spinach (3). However, because chlorine is used in some spinach prepackaging plants, it seemed desirable to make further tests to determine whether chlorination of washing water would reduce the amount of decay in the prepackaged spinach.

Compounds used were chloramine (formed by the interaction of chlorine gas and an ammonium salt) at a concentration of 73 p.p.m. available chlorine, sodium hypochlorite powder (5 p.p.m. available chlorine), and succinchlorimide (41–107 p.p.m. available chlorine). The concentration of available chlorine was determined iodometrically with standard sodium thiosulphate. In one test the pH of the treatment water, determined colorimetrically, was 6.2 before and after the addition of succinchlorimide (107 p.p.m. available chlorine). In no other instance was the hydrogen-ion concentration taken. Spinach washed with untreated water and prepackaged at the same time served as a control. In some of the commercial prepackaging plants detergents (sodium-alkyl-benzene sulfonate and alkyl-aryl-sodium sulfonate) have been placed in the water to improve soil removal. The numbers of bacteria in the wash water and on spinach leaves and the amount of decay and salability of the prepackaged product were determined.

Chlorine markedly reduced the numbers of bacteria in the wash water. In one test untreated water contained 1.92×10^6 bacteria per ml., whereas water treated with sodium hypochlorite in quantities to yield 5 p.p.m. available chlorine had 1400 bacteria per ml. Spinach leaves washed in chlorinated water consistently had fewer bacteria than untreated leaves, but large numbers of bacteria still remained on the treated leaves (Table 1). Observations of bagged spinach washed with untreated or with chlorinated

[1] Report of a study made under the Research and Marketing Act of 1946, RMA 44.

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water showed no apparent difference in decay or in length of shelf-life. The results of one test are in table 1.

TABLE 1.—*The effect of chlorination on bacterial count, decay, and salability of prepackaged spinach held at 68° F.*

Treatment of wash water	Total weight of spinach (in gm.)[a]	Percentage of spinach			Salability[c]
		Sound	Light decay	Heavy[b] decay	
One day after prepackaging[d]					
Not treated	614	95.6	4.4	0.0	4 S
Detergent, 0.1 per cent	565	90.8	9.2	0.0	4 S
Chlorine, 107 p.p.m.	561	96.3	2.8	0.9	4 S
Chlorine, 97 p.p.m. plus detergent, 0.1 per cent	538	93.3	6.5	0.2	4 S
Two days after prepackaging[d]					
Not treated	1132	66.7	21.5	11.8	3 B, 7 U
Detergent, 0.1 per cent	1115	74.7	16.8	8.5	2 B, 8 U
Chlorine, 107 p.p.m.	1127	68.9	24.5	6.6	4 B, 6 U
Chlorine, 97 p.p.m. plus detergent, 0.1 per cent	1089	57.7	27.0	15.3	1 S, 2 B, 7 U

[a] Initial bacterial counts for two bags of spinach from each of the respective treatments were 13.4, 9.0, 0.9, and 0.5 million bacteria per gm. spinach.

[b] Leaves with light decay would ordinarily be utilized after the decay spots were removed; those with heavy decay would be discarded.

[c] Four bags from each of the respective treatments used for salability data one day after prepackaging; ten bags, two days after prepackaging. Bags rated as salable (S), barely salable (B) or unsalable (U).

[d] Two bags used for weight and decay data 1 day after prepackaging; four bags used for similar data 2 days after prepackaging.

SILVER-TREATED CELLOPHANE

Silver in various forms (silver nitrate, colloidal silver iodide, and protein-silver preparations) is known to have bactericidal properties (4). Bags made of 450 MSBO Cellophane, containing less than 0.2 per cent silver ion as the active ingredient, were available for tests.[3] The antibacterial property of the treated Cellophane was demonstrated by the formation of growth-free zones around pieces of film on nutrient agar plates planted with the common soft-rot bacterium (*Erwinia carotovora*). Because it was thought that the ionic silver might become sufficiently dispersed in the films of water on the leaves and on the Cellophane to reduce decay and prolong the shelf-life of the spinach, four tests were made with spinach packed in these bags.

At the start of each test the initial bacterial count was high (½ million to 45 million bacteria per gm. of spinach leaf tissue). When spinach was held in bags of untreated film at 68° F. the counts rapidly increased to from 311 million to 9.8 billion within 2 days. On spinach leaves held in the treated Cellophane bags the increase in bacteria was less rapid. Bacterial counts for spinach in treated bags held for 3 days at 45° F. were much the same as the initial counts, but there was some increase in the untreated bags held at this temperature. The results of one representative test are in table 2. In another of the four tests the spinach was first washed

[3] Bags supplied by the Chloramine Co., New York, N. Y.

with water containing 103 p.p.m. available chlorine plus 0.1 per cent detergent to reduce the initial bacterial load of the spinach before packaging in the silver-treated Cellophane. The results of the holding tests were, however, similar to those in table 2.

In all four tests with spinach prepackaged in 284 Cellophane bags, that from silver-treated and untreated bags had about the same amount of decay.

HOLDING TEMPERATURES AFTER PREPACKAGING

During the tests it was observed that decay was markedly less and shelf-life was extended when prepackaged spinach was held at reduced temperatures. Smith (5) has reported that bacterial soft rot of spinach is caused by *Erwinia carotovora* which induces a rapid decay under conditions of high humidity and high temperature (75° to 86° F.), and that it is held in check by either low temperature (40° F.) or low humidity.

Holding tests were made at several temperatures with bags made of various films. When the leaves were examined for decay, bags of spinach were rated as salable, barely salable, or unsalable, on the basis of the amount of condensation and fogging of the film, presence of free water in bags, limpness of the package when squeezed, bad odor upon opening, wilting of leaves, leaf water-soaking, abnormal flavor in raw or cooked leaves, and amount of sliminess and decay. For the 1,165 bags prepackaged and stored, the approximate time during which the spinach remained salable was: 7–8 days at 33° F. (67 bags); 4–7 days at 39° F. (24 bags); 3–6 days at 45° F. (380 bags); 3–4 days at 49° F. (24 bags); 1–2 days at 68° F. (589 bags); and 1–2 days at 73° F. (81 bags). An occasional bag was still salable on the 14th day at 33° F., on the 11th day at 39° F., on the 10th day at 45° F., and on the 3rd day at 68° F. Reduction of the holding temperature from 68° or 73° F. to 33° F. extended the shelf-life of prepackaged spinach by about 6 days.

TABLE 2.—*Comparison of decay, salability, and bacterial count of spinach prepackaged in silver-treated and nontreated Cellophane bags*

Treatment of spinach	Number of bags examined—total weight of spinach (in gm.)	Percentage of spinach			Number of bacteria on spinach leaves (in millions per gm.)
		Sound	Light decay	Heavy decay	
Untreated bags					
Initial data	12 — 3379	83.5	16.5	0.0	11.4
1 day (68° F.)	11 — 3044	59.1	38.1	2.8	760.6
2 days (68° F.)	17 — 2697	17.3	53.3	29.4	2262.4
3 days (45° F.)	8 — 2245	57.8	38.8	3.4	19.3
Silver-treated bags					
1 day (68° F.)	11 — 2991	90.3	8.4	1.3	377.2
2 days (68° F.)	17 — 2629	20.5	49.9	29.6	1272.0
3 days (45° F.)	9 — 2555	55.7	41.4	2.9	8.0

A method by which spinach temperatures could be quickly reduced after packaging would aid in extending the shelf-life of the product. Experiments demonstrated that prepackaged spinach in sealed but perforated bags

packed in perforated shipping cartons could be cooled from 79° F. to 32° F. at the center of the bags in about 5 min. in a vacuum chamber under a vacuum of 4.5 mm. mercury (1). Vacuum cooling prior to shipment or storage is an effective means of rapidly reducing the commodity temperature after the packaging operations.

CENTRIFUGAL DRYING FOLLOWING WASHING

The soft-rot organism *Erwinia carotovora* grows most rapidly at relatively high temperatures and high humidities; and spinach-leaf decay may be reduced if diseased leaves are removed to dry air (5). In spinach packing plants in the New York City area different types of laundry centrifuges (called "extractors" in the trade) have been used to remove water from spinach after the washing operation. These had different rates of speed and load capacity. In some plants they were run for as little as ½ min.; in others, for as long as 9 min. Thus the spinach was packed relatively wet in some packing plants and comparatively dry in others. The following tests were made to determine the effect of time of centrifuging upon the water loss from wet spinach, on the subsequent decay, and on the shelf-life of prepackaged spinach.

In the first test three lots of spinach weighing 71.3, 70.2, and 72.5 lb., respectively were centrifuged for ½, 1, and 4 min. Each group was bagged separately, and 12 prepackaged bags of each treatment were held at 45° and at 68° F. The weight loss after ½, 1, and 4 min. of centrifuging was 13.2, 21.8, and 27.2 per cent, respectively. Observations made immediately after packaging and after storage at 68° F. showed that longer centrifuging resulted in drier spinach, less decay, and longer shelf-life. An additional test was run at the same plant, in which the periods of centrifuging were 1, 2, and 3 min. Because of a number of factors, such as the time the bags of washed spinach were allowed to stand on the floor before centrifugation, the weight of the load in the centrifuge, and probably other factors, these three lots of spinach lost practically the same percentage of water, namely 21.1, 21.2, and 19.1, in spite of the fact that they were centrifuged for varying periods of time. As a result, no apparent differences in the decay, appearance, or salability of the three lots of prepackaged spinach were noted.

The following tests were performed at two different packing plants. The first test was run during a period of hot weather. One lot of wet spinach was centrifuged ½ min. and lost 7.5 per cent of its original weight; the second lot, centrifuged 3½ min. lost 19.1 per cent. The drier spinach developed less decay than the wet spinach on both the first and second day and remained in a salable condition for one additional day (Table 3). The second test was made during cool weather at a different packing plant, where the spinach was normally being centrifuged approximately 6–9 min. One lot was centrifuged 5 min. and lost 28.2 per cent of its weight; a second lot, centrifuged 10 min., lost 28.7 per cent. No differences were noted in the amount of decay or length of shelf-life (Table 3).

In none of the tests was there an increase in the amount of mechanical injury to the spinach centrifuged for the longer periods.

With longer centrifugation, the spinach leaves were drier and less water-soaked, less decay occurred in the packages, and the shelf-life was longer.

SUMMARY AND CONCLUSIONS

Bacterial soft rot is the principal cause of decay and reduction in shelf-life of prepackaged spinach. Although inorganic and organic chlorine compounds reduced the numbers of bacteria in the wash water and on spinach leaves, chlorination of the wash water did not decrease decay or increase shelf-life of the prepackaged product. Packing spinach in silver-treated Cellophane bags did not significantly control decay or lengthen shelf-life. More thorough centrifugal drying of washed spinach reduced decay and lengthened shelf-life of the prepackaged product. Refrigeration of prepackaged spinach was the best means of controlling decay and increasing shelf-life.

TABLE 3.—*Effect of the amount of centrifuging on drying, decay, and salability of prepackaged spinach stored at 68° F.*

Centrifuging time and percentage of weight lost, and holding time	Number of bags examined—total weight of spinach (in gm.)	Percentage of spinach			Salability[a]
		Sound	Light decay	Heavy decay	
0.5 min.; 7.5					
0 days	2 — 564	91.2	8.8	0.0	2 S
1 day	8 — 2326	56.4	37.1	6.5	6 B, 2 U
2 days	11 — 3137	27.5	30.7	41.8	11 U
3.5 min.; 19.1					
0 days	2 — 567	89.8	10.2	0.0	2 S
1 day	8 — 2188	70.0	27.1	2.9	7 S, 1 B
2 days	13 — 3619	52.5	27.9	19.6	13 U
5.0 min.; 28.2					
0 days	9 — 2449	88.8	11.0	0.2	9 S
1 day	7 — 1936	82.7	16.8	0.5	7 S
2 days	13 — 3442	53.1	33.6	13.3	13 U
3 days	7 — 1836	29.6	37.1	33.3	7 U
10.0 min.; 28.7					
0 days	9 — 2458	90.6	8.9	0.5	9 S
1 day	7 — 1915	82.5	16.8	0.7	7 S
2 days	13 — 3482	60.3	26.9	12.8	13 U
3 days	7 — 1831	39.2	36.3	24.5	7 U

[a] Salable (S), barely salable (B), or unsalable (U).

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PHYSIOLOGICAL RACES OF THE CROTALARIA WILT FUSARIUM[1]

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(Accepted for publication March 12, 1951)

Fusarium wilt of crotalaria has been known as a destructive disease in India (7, 10, 11, 12, 20) and other regions (5, 8, 17, 19, 21, 23) for many years, and recently it has also been found in Brazil (9). The first report of its occurrence in the United States was in 1945 by Bain (4) who found affected crotalaria plants in tung orchards in Louisiana and Mississippi. He further reported that in pot experiments the L. Z. soybean was susceptible to the disease. Since physiological races of the cowpea-soybean wilt *Fusarium* (2) had been discovered, it seemed desirable to obtain cultures of the fungus for comparative studies. When no wilting plants were observed in a survey of numerous fields of crotalaria in South Carolina, a collection was made in Louisiana and Mississippi. Several Fusarium isolates from this material and other isolates from Florida[3] and India[4] were used in the experiments here reported. The wilt in Florida has been reported (3).

METHODS

Crotalaria seed were treated with concentrated H_2SO_4 to assure prompt germination, and planted in pots of steamed sand in a greenhouse. Plants were inoculated when about 21 days old, except those of *C. juncea* L. which were about 10 days old, by methods which have been described (1). Usually the roots were cut and 500 ml. of a 3-day-old solution culture of the fungus were poured around the plants. In the tests, when young plants were removed from the pot and replanted after the roots had been washed and dipped in the inoculum, a paper shade was put over each pot until the plants were well established; otherwise they did not withstand the shock of transplanting, and it was impossible to record accurately the effect of the wilt organisms. When this precaution was taken, however, the results of the two methods were essentially the same and have been combined in the tables. Plants were removed from the pots when definite external symptoms of wilt were apparent, and sections of some of the stems were plated to recover the fungus. The experiments were usually terminated in 2 months. Each series contained pots of noninoculated plants of the varieties used.

[1] Contribution of the Department of Botany and Bacteriology, in cooperation with the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture. Technical contribution No. 188, South Carolina Agricultural Experiment Station. Acknowledgment is given to C. C. Bennett for aid in certain phases of the greenhouse investigations.

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[3] Culture supplied by J. L. Weimer.

[4] Cultures supplied by R. S. Vasudeva and M. K. Patel.

Isolations of the wilt *Fusarium* were made from plants of *C. spectabilis* Roth and *C. intermedia* Kotschy collected in the Louisiana-Mississippi area. Six single-spore lines from these isolates were used in the inoculations, together with four from the Indian isolates and one from the Florida isolate. Occasionally the mass cultures were included for comparisons.

The following species of crotalaria[5] were inoculated with the crotalaria wilt *Fusarium: C. juncea* L., *C. spectabilis* Roth, *C. verrucosa* L., *C. lanceolata* E. Mey., *C. intermedia* Kotschy, *C. incana* L., *C. mucronata* Desv. var. Giant Striata, and *C. usaramoensis* Bakerf. Some of these were also inoculated with wilt Fusaria from pigeon pea[6] (*Cajanus cajan* (L.) Millsp.), soybean (*Soja max* (L.) Sieb. & Zucc.), cowpea (*Vigna sinensis* (Torner) Hassk.), okra (*Hibiscus esculentus* L.), cotton (*Gossypium hirsutum* L., *G. barbadense* L., *G. herbaceum* L.), tobacco (*Nicotiana tabacum* L.), and tomato (*Lycopersicon esculentum* Mill.). The crotalaria wilt *Fusarium* likewise was used to inoculate plants of a number of genera which are listed later.

TABLE 1.—*Results of inoculations of crotalaria with Fusarium-wilt isolates from crotalaria*

Species	Crotalaria isolates								
	India			Louisiana–Mississippi			Florida		
	No. of plants	Percentage of plants with symptoms of wilt		No. of plants	Percentage of plants with symptoms of wilt		No. of plants	Percentage of plants with symptoms of wilt	
		External	Internal only		External	Internal only		External	Internal only
C. juncea	134	86.6	9.7	126	31.8	48.4	18	50.0	27.8
C. spectabilis	181	79.6	5.0	148	87.8	10.8	48	93.4	0.0
C. verrucosa	17	70.6	17.6	37	94.6	2.7	19	78.9	0.0
C. incana	117	0.9[a]	11.9	114	97.4	0.9	38	100.0	0.0
C. intermedia	205	0.0	2.0	362	95.3	2.5	43	93.0	7.0
C. mucronata	33	0.0	9.1	37	67.6	18.9	.....	.....	.....
C. mucronata var. Giant Striata	141	0.0	17.7[a]	224	90.6	4.0	30	100.0	0.0
C. lanceolata	221	71.5	20.8	190	2.1[a]	24.2[a]	45	97.8	0.0
C. usaramoensis	11	0.0	0.0	8	0.0	87.5	15	0.0	100.0[a]

[a] Very slight symptoms.

RESULTS

Inoculations of Crotalaria with Wilt Fusaria from Crotalaria

Typical wilt symptoms developed in the diseased plants in 10 to 21 days depending upon the host and the isolate. When sections of some of these stems were surface-sterilized and plated on water agar, the wilt fungus

[5] Seed were supplied by Roland McKee and G. E. Ritchie of the U. S. Department of Agriculture, and J. A. Riley of the Sand Hill Experiment Station, Columbia, S. C.

[6] Isolation made from a diseased stem sent by R. P. Asthana.

could be recovered. Since there were no significant differences in pathogenicity among the Louisiana-Mississippi isolates or among the Indian isolates, the results for each group have been combined in table 1. It will be seen in this table that there are three physiological races of the crotalaria wilt *Fusarium*. The Indian isolates representing race 1 caused wilt of all species except *C. mucronata, C. intermedia, C. incana,* and *C. usaramoensis;* the Louisiana-Mississippi isolates representing race 2, of all except *C. lanceolata* and *C. usaramoensis;* and the Florida isolate representing race 3, of all except *C. usaramoensis.* Since all isolates caused wilt of *C. juncea, C. spectabilis,* and *C. verrucosa,* and no wilt of *C. usaramoensis,* the races cannot be differentiated by these species. Only one differential host, *C. lanceolata,* will separate the races in the United States, yet this difference

FIG. 1. The reactions of (upper row) *Crotalaria lanceolata* and (lower row) *C. intermedia* to Fusarium isolates from Florida (left), Louisiana-Mississippi (center), and India (right). Healthy plants at center and right, respectively.

in pathogenicity is distinct. The Florida isolate was very virulent on this species, whereas the Louisiana-Mississippi isolates in 12 tests, involving 122 plants, produced slight external symptoms of the disease in only four plants. Furthermore, 77 of the remainder had no discoloration in the stem and the others had only a slight streak. The reaction of *C. lanceolata* to the three races is shown in figure 1 (upper). In figure 1 (lower) it will be seen that the Indian isolates do not cause wilt of *C. intermedia,* hence the two species can be used to separate the three races of the pathogen. *C. mucronata* or *C. incana* along with *C. lanceolata* would also serve the same purpose. Throughout the experiments, except in several instances

with the Florida isolate, there were a few healthy plants among the diseased ones, indicating that wilt-resistant lines can probably be selected.

The results of other workers indicate that either there are additional races of the fungus, or some species of crotalaria vary in disease resistance. Since the isolates used by Joffily (9) did not cause wilt of *C. juncea* and *C. verrucosa,* they are probably different from those studied by the authors. Vincens (22) observed a wilt of *C. usaramoensis* in Saigon. Uppal and Kulkarni (20) reported that in the field this species had wilt symptoms at the end of the season, and in pots after 4 months. However, in limited tests of the present investigation, inoculated plants of this species grew vigorously and had no external symptoms of wilt at the end of 2 months. There was no discoloration of the stele of the plants inoculated with an Indian isolate, but 95 per cent of those inoculated with an isolate of each race in the United States had slight internal discoloration. With such slight symptoms, it seems unlikely that these plants would ever have wilted.

Padwick et al. (15) did not observe physiological races of the crotalaria wilt *Fusarium,* but these would not have been evident since only one species, *C. juncea,* was used for the isolations and inoculations.

Inoculations of Crotalaria with Wilt Fusaria from Other Hosts

Inoculations of crotalaria with wilt Fusaria from other hosts were made as follows: all species but *C. usaramoensis* with an isolate from pigeon pea; *C. spectabilis, C. juncea,* and *C. mucronata* with isolates of races 1 and 2 from cowpea-soybean; only *C. spectabilis* with isolates from okra and tomato and from Indian, Egyptian, and U. S. cotton. The virulence of all the cultures had been recently proved. There were no external symptoms of wilt in any case. Other workers (5, 9, 15, 20) have also inoculated species of crotalaria with some of these wilt Fusaria but no wilting occurred.

Inoculation of Other Plants with the Crotalaria Wilt Fusarium

From table 2, it can be seen that one or more of the crotalaria isolates produced no symptoms of wilt on Criollo variety of sesame (*Sesamum indicum* L.), Porto Rico sweet potato (*Ipomoea batatas* (L.) Lam.), Burley tobacco, Hurley's Special Rowden cotton, Sakel cotton, and red, white, and No. 69[7] pigeon peas. It is of interest, however, that one of the Louisiana-Mississippi isolates produced appreciable internal discoloration in cowpeas and other isolates produced even greater discoloration in the Cassia (*Cassia tora* L.) plants. The discoloration in the cowpeas was comparable to that produced by the races of the cowpea wilt *Fusarium* to which they are resistant (2). Other workers (5, 9, 15, 19) have recorded negative results when some of these plants, as well as beans, were inoculated with the crotalaria wilt *Fusarium.*

Only a small percentage of the L. Z. and Yelredo soybean plants had any external symptoms of wilt, the former 14.3 per cent with the Florida

[7] Seed of No. 69 supplied by B. P. Pal.

isolate and the latter 11.5 per cent with the Louisiana-Mississippi isolates. However, 40.9 per cent of the Yelredo plants also had internal wilt symptoms with the Louisiana-Mississippi isolates. Bain (4) reported no detailed data, but one can infer that he obtained much more wilting of the L. Z. soybean with his isolates.

The Causal Organism

No comprehensive discussion of all the literature on this fungus, nor a lengthy description of the cultures which were used in this investigation, will be attempted. Padwick (13) made a careful study of the taxonomy and morphology of the fungi causing wilt of crotalaria and pigeon pea and

TABLE 2.—*Results of inoculations of plants other than crotalaria with the Fusarium-wilt isolates from crotalaria*

Plants inoculated	Crotalaria isolates								
	India			Louisiana–Mississippi			Florida		
	Number of plants with symptoms								
	External	Internal only	None	External	Internal only	None	External	Internal only	None
Soybean:									
Yelredo	0	1	41	7	25	29	0	1	19
Yelnando	0	0	20	0	1	9	..	..	..
L. Z.	0	0	40	0	0	56	2	0	12
Cassia tora	0	4	10	0	19	11	..	..	..
Cowpea:									
Lady Finger	0	0	20	0	2	9	..	..	..
Sumptuous	0	3	17	0	3	7	..	..	..
Brown Sugar Crowder	0	0	20	0	8	2	..	..	..
Pigeon pea:									
White	..	..	..	0	0	10	..	..	..
Red	..	..	..	0	0	10	..	..	..
69	0	0	39	0	0	20	0	0	10
Cotton:									
Rowden	0	0	10	0	0	10	..	..	..
Sakel	0	0	10	0	0	10	..	..	..
Burley tobacco	0	0	5	0	0	5	..	..	..
Sweet potato	0	0	5	0	0	6	..	..	..
Sesame	..	..	..	..	..	..	0	0	9

compared them with forms of *F. vasinfectum* Atk. and *F. lateritium* Nees var. *uncinatum* Wr. (22) obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland. He was unable to distinguish the wilt *Fusarium* of crotalaria from that of the pigeon pea either culturally or morphologically. On the basis of their pathogenicity, he designated the former *F. udum* var. *crotalariae* and the latter *F. udum* var. *cajani*, but he (14) later decided that the pigeon-pea Fusarium should be called *F. udum* since the original description of the species included mention of its pathogenicity on this host. He (13) also showed that *F. lateritium* Nees var. *uncinatum*

Wr. is a synonym of *F. udum* Butl. and stated that *F. vasinfectum* could be distinguished from these forms morphologically and culturally.

The relatively few crotalaria-wilt isolates that have been available for this study fall into the range of variations described by Padwick (13). They were grown on rice, potato-dextrose agar, and crotalaria stems. The colors are reported according to Ridgway's (16) nomenclature. The Indian isolates were mycelial and pionnotal forms which produced bright yellow and orange pigments, terminal and intercalary chlamydospores, abundant microspores, and many long, slender, hooked macrospores. The liquid inoculum of these isolates was pale green-yellow, which the authors have never observed with any of the many Elegans Fusaria cultured by this method. The U. S. isolates when first obtained were fluffy mycelial ones producing abundant microspores but few macrospores. Occasionally a long, decidedly hooked macrospore could be found, but usually they were short with the apical cell tapered or only slightly hooked, resembling those of the section Elegans. The Louisiana-Mississippi isolates produced numerous blue-black sclerotia and abundant terminal and intercalary chlamydospores, the latter often in groups and chains; the Florida isolate, no sclerotia and few chlamydospores. The bright yellow and orange colors originally described for *F. udum* and produced by the Indian isolates were lacking, but other tints appeared on rice or potato-dextrose agar when the cultures were kept in daylight. The growth and colors resembled those of many of the hundreds of isolates of the section Elegans that have been observed by the authors. The Florida isolate produced only a salmon-pink pigment, but the Louisiana-Mississippi isolates produced a wide range of colors including shades of purple, vinaceous, coral pink, buff, and dull dark blue. During subsequent transfers of the U. S. cultures, pionnotal forms have arisen which produced the bright yellow and orange colors on the solid media and also the green yellow in the liquid inoculum. Large hooked macrospores, smaller than those of the Indian isolates, have also appeared, especially on crotalaria stems. Thus, no morphological or cultural basis has been found for the separation of the physiological races.

The difficulties of deciding whether *F. udum* var. *crotalariae* belongs to the section Lateritium or Elegans are discussed fully by Padwick (13). All the observations of the present study also indicate that this highly variable *Fusarium* is an intermediate form. However, the decidedly hooked spores and the brilliant yellow and orange pigments which are characteristic of Lateritium, but not of Elegans, are found in *F. udum* var. *crotalariae*. Snyder and Hansen (18) have named it *F. oxysporum* Schl. f. *udum*, but, as Padwick pointed out, this classification was made without an examination of the fungus. Joffily (9) sent cultures to Carrera (6), who named the Brazilian fungus *Fusarium oxysporum* Schl. f. *crotalariae* n. f. From Carrera's description of the macrospores as long, diameter rather uniform, and the apex slightly hooked, as well as from the colors of his cultures, some of

which occur chiefly in *F. udum*, one is led to believe that Carrera was probably dealing with this fungus.

SUMMARY

Cultures of the crotalaria wilt *Fusarium* were obtained from the Louisiana-Mississippi area, from Florida, and from India. Inoculations of eight species of crotalaria were made in the greenhouse with one or more isolates from each area.

On the basis of pathogenicity to species of crotalaria, three physiological races were found; race 1 from India, race 2 from the Louisiana-Mississippi area, and race 3 from Florida.

Wilt Fusaria from cotton, okra, tomato, pigeon pea, and races 1 and 2 from cowpea-soybean were used to inoculate one or more species of crotalaria. There were no external symptoms of wilt in any case.

Inoculations with one or more of the crotalaria-wilt isolates produced no symptoms of wilt on sesame, Porto Rico sweet potato, Burley tobacco, Rowden and Sakel cotton, and three varieties of pigeon peas. One isolate produced appreciable internal discoloration in 42 per cent of cowpeas, and other isolates produced even greater discoloration in 90 per cent of the Cassia plants.

Only the Louisiana-Mississippi isolates caused 11.5 per cent wilt of the Yelredo soybean, and only the Florida isolate caused 14.3 per cent wilt of the L. Z. soybean.

There were appreciable differences in the morphological and cultural characters of the Indian and American isolates; however, they fall within the range of *Fusarium udum* var. *crotalariae* Padwick, and this name is accepted for the American fungus.

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TESTS OF CRANBERRY VARIETIES AND SEEDLINGS FOR RESISTANCE TO THE LEAFHOPPER VECTOR OF FALSE-BLOSSOM DISEASE[1]

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(Accepted for publication March 15, 1951)

INTRODUCTION

The most serious disease of the cranberry (*Vaccinium macrocarpon* Ait.) throughout most of its cultivated range is false blossom, caused by a virus (*Chlorogenus vaccinii* Holmes) and transmitted by the blunt-nosed cranberry leafhopper, *Scleroracus vaccinii* (Van D.) (4). Although the rate of spread in the field is relatively slow on certain cranberry varieties, no variety has been resistant to infection by the virus when caged with viruliferous leafhoppers. Wilcox and Beckwith (7) have indicated a negative correlation between the rate of spread of the disease and the relative attractiveness of a variety as a food plant for the vector, which suggests that "field resistance" to false blossom actually consists in resistance to attack by the insect.[2] No recent evidence is at variance with this conclusion. The resistance of the McFarlin and Early Black varieties has been of practical importance to cranberry growers. Huber and Schwartze (5) have shown that analogous "resistance" in red raspberries may be transmitted to the progeny of a resistant parent.

The knowledge of varietal differences led to the breeding of cranberries to obtain varieties more resistant than those at hand. Bain (2) in Wisconsin and Bergman (3) in Massachusetts grew several thousand hybrid seedlings from crosses of the major commercial varieties in the respective areas. Beckwith obtained 112 seedlings from a cross of Early Black and McFarlin which he placed with Bain's and Bergman's in a preliminary test in New Jersey. Of the 1,800 seedlings in the Whitesbog, New Jersey, test set out in 1934, about half were hybrids of McFarlin × Early Black; the rest were of various parentages. The plants began to fruit in 1938 and records were made of their yields, as well as of fruit and vine characters, in 1938, 1939, and 1940. During the summer of 1940 the writer made selective feeding tests with more than 100 of the seedlings that appeared most promising

[1] This paper was prepared by R. B. Wilcox before his death October 22, 1949. It had been written some years previously and revised slightly from time to time and had been reviewed by the late N. E. Stevens. This has since been reviewed by H. F. Bain, formerly senior pathologist, H. F. Bergman, senior pathologist, E. J. Koch, assistant biometrician, and G. M. Darrow, principal horticulturist, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and F. B. Chandler, research professor of cranberry culture, Massachusetts Agricultural Experiment Station. A summary of the work by J. M. Delap under Wilcox's direction is given on page 12 and in table 3.

R. B. Wilcox was associate pathologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

[2] Throughout the present discussion susceptibility and resistance have reference to liability to attack by the vector rather than to reaction to the virus.

horticulturally, to determine their probable reaction to false blossom. Most of these early seedlings were discarded after selections were made in 1940.

The present paper records the results of the selective feeding tests on these varieties and hybrids, reports the effects of variations in technique, and especially evaluates the tests as an indicator of resistance to vector feeding.

MATERIALS AND PROCEDURES

The selective feeding test as described by Wilcox and Beckwith (7) in 1930 was repeated in 1931 and 1932 with only minor modifications. The leafhoppers were collected from cranberry bogs but not from varieties under test. In the spring of 1940, an experimental building was erected at the edge of the test bog, with screened windows from a height of 3 ft. to the eaves (Fig. 1). Tables wide enough to hold two rows of cages were

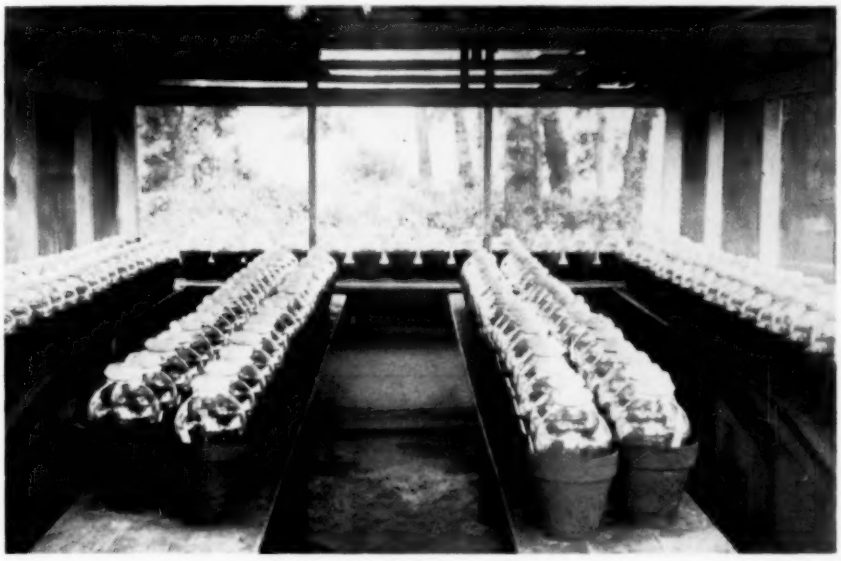

FIG. 1. View of the interior of the building used in 1945 for tests of susceptibility of cranberry seedlings to leafhopper feeding. In each pot were cuttings of 19 different varieties or seedlings. Three of these were always Howes, Early Black, and Shaw's Success, used as standards for comparison.

built against the outer walls and through the center. Six-inch flower pots were nearly filled with bog soil, and then ¼ in. of white sand was added. Healthy cranberry uprights (cuttings) were "planted" in these pots and the soil was kept moist to maintain them in vigorous condition. The lantern globes capped with cheesecloth used in the earlier work were replaced by newly designed globes of somewhat larger diameter, shorter, and with a prominent collar at the top. Three cuttings of each of seven varieties or numbered hybrid seedlings were placed in each globe-cage. Six of the varieties were regularly spaced near the circumference and the seventh occupied the center. Seven cages, each containing the same varieties, constituted a series. A label identifying the cages was placed at position 1 and

the varieties were rotated one place in setting up each successive cage in the series, so that when the labels were all in the same direction each variety occupied all seven positions in respect to the source of light, and the "light positions" were alike in all cages in relation to the labels. Thirty-five blunt-nosed leafhoppers collected from *Chamaedaphne calyculata* (L.) Moench. were introduced into each cage. From 10 to 20 successive daily readings of a series were made, the number of insects on each variety being recorded as well as the number on the glass surface. Upon completion of a reading each cage was lifted, jarred or knocked against the table, and returned to the original position. In 10 days the insects appeared to predominate on the side of the cage exposed to the strongest illumination, indicating that choice of position was being made on the basis of light rather than variety. An attempt was then made to stimulate movement by disturbing all insects with a slender brush after each reading. To learn the effect of this treatment as soon as possible, readings were made once before midmorning and once in late afternoon until nine readings had been completed. At this time it was decided that such disturbance had further stimulated choice of position by light rather than by variety. Thereafter, all cages were turned 60 degrees clockwise after each reading.

RESULTS

The scale of susceptibility. Of the 47 series of seven cages each tested in 1940, all but four included both Howes and McFarlin varieties. The 835 uprights of each of these standards constituted a representative sample of these varieties under New Jersey conditions. More than 4,000 observations had been made, during which 18,324 "neutral" insects were recorded on Howes and 13,754 on McFarlin, or a ratio of about 4 to 3. Considering the aggregate of all series as a single test, the difference in insect number between Howes and McFarlin was $4{,}570 \pm 179$.[3]

The ratio of 4 : 3 fails to take into account the possibility that the insects counted on a variety are not necessarily all feeding there, and the probability that some insects in a cage would be on a non-food plant. In nearly all observations a few of the insects were located elsewhere than on the plants, i.e., usually on the inner surface of the globe; hence they were recorded as "on glass."

Blisard (1), working under supervision of the writer in 1931, tested 26 local species of possible hosts for *Scleroracus vaccinii* by confining a number of the insects in a cage containing one species of plant and observing the length of life of the insects (Table 1). At each observation he recorded the number of live insects on the plant and "on glass." After the selective tests of 1940, a reanalysis of Blisard's results revealed an unsuspected correlation between the suitability of a plant for food, as shown by longevity,

[3] The frequencies of 0, 1, 2 . . . . . n insects on varieties in all series tested agreed well with the Poisson distribution, so the same formula was used as in 1930 (7) for testing the significance of a difference between two varieties; namely, $a - b \pm \sqrt{a + b}$, in which a and b are the respective totals of insects observed on the two varieties in all observations.

and the proportion of insects found on the plant. It was to be expected that, for food plants, most of the vectors would be found on the plant, and only when the nutritive value of a plant was so low that the insects could not long survive were more vectors likely to be found elsewhere than on the plant. A point of chief interest is that, in their search for food, or in an attempt to feed, considerable proportions of the insects were found not only on plants incapable of sustaining life, but even on those which appeared to be slightly toxic.

On the basis of Blisard's results, if a series of cages was prepared containing a single variety of cranberry or other species, and a number of readings showed as many insects on the glass as on the plants, it could be concluded with some assurance that the variety or species in the cages was not a food plant for the insects, and was therefore immune from their attack.

The number of insects counted on the glass in 1940 was consistently about one-fourth the number counted on McFarlin and was subject to some independent day-to-day variation, to some series-to-series variation, and to some variation inverse to that on McFarlin, such as would follow any physical

TABLE 1.—*Proportions of leafhoppers, Scleroracus vaccinii (Van D.), found on plants in cages each containing a single plant species, and longevity of such leafhoppers (adapted from Blisard)*

Host group	Number of species	Longevity of insects		Insect positions	
		General mean	Average maximum per species	Total	On plant
		Days	*Days*	*No.*	*Pct.*
Ericaceous food plants (*Chamaedaphne, Leucothoe*)	2	24.5[a]	37.0[a]	1,251	85
Ericaceous plants to which a few insects adapted themselves (*Gaylussacia, Leiophyllum*)	4	7.4[a]	27.0[a]	750	61
Ericaceous non-food plants (including *Kalmia*)	10	3.9	6.8	815	49
Other non-food plants	7	3.5	6.9	422	44
Slightly toxic plants, non-ericaceous (*Carex, Comptonia, Juncus*)	3	1.8	3.4	114	27
Checks, no plant		2.9	6.0	164	.....

[a] Incomplete: either some insects were living at time of last observation, or experiment was terminated by death of the host plant, or both.

disturbance. For that reason the number of insects on the glass could not be used as direct measure of the attractiveness of varieties under test so as to avoid the necessity for standard varieties. A scale of susceptibility was adopted on which the zero point was represented by 25 per cent of the insects normally found on McFarlin, and McFarlin itself was assigned an arbitrary value of 13. With the points 13 and 0 on the scale already determined by the number of insects on McFarlin, the susceptibility rating of any other variety was calculated by the formula $\sqrt{676 \frac{N_x}{N_m}} - 13 = S$, in which N_m is the total number of insects counted on McFarlin, N_x is the correspond-

ing number found on the variety under trial, and S is the position of the variety on the scale of susceptibility.

On only one occasion (in 1931) was one of Blisard's non-food plants ever included with cranberries in a selective test. This was *Kalmia angustifolia* L. In Blisard's test 49 per cent of the insects had been recorded on the plant and these had lived an average of 3.9 days with a maximum of 7 days. In the selective test, 80 individuals were found on *Kalmia* and 348 on McFarlin. The above formula gave to *Kalmia* an S rating of -0.5 ± 1.0, which is satisfactorily close to zero.

Application of the formula to the results of all comparative tests gave the Howes variety an S value of 17.01 ± 0.16. The rating of an unknown variety could then with equal propriety be determined from the Howes by substituting the number on Howes (N_h) for (N_m) in the formula and using the coefficient 900.6.

Since the sum of the two independent but variable standard varieties—Howes and McFarlin—was subject to less variation than either variety alone, the combined proportion of insects counted on Howes and McFarlin was used as a standard for estimating the susceptibility of other varieties. Therefore, the formula used in obtaining the S values reported in this paper was

$$\sqrt{1576.6 \frac{N_x}{N_m + N_h}} - 13 = S.$$

Any wide deviation of N_h/N_m from the normal ratio was taken to indicate an unduly large error in sampling, and in a few cases this was sufficient to invalidate the results of an entire series. It was later discovered that this ratio varied with the average attractiveness of the other varieties in the series ($\overline{S_x}$). If all the "unknowns" were very attractive, the number of insects choosing McFarlin was abnormally low, and N_h/N_m was consequently high. On the other hand, if all of the "unknowns" were approximately as unattractive as McFarlin, the number selecting Howes was not correspondingly increased and fewer differences appeared between Howes and McFarlin. The regression of N_h/N_m on $\overline{S_x}$ was not a straight line function and its formula was not determined.

Susceptibility of named varieties. The selective test was applied in 1931 to the named varieties, Cherry Red, Garwood Bell, Howard Bell, Plum, Richards, and Woolman, which had arisen as selections from wild vines in New Jersey, and most of which were grown only locally in the bogs of the originators. The variety Taylor was tested in 1941. Plum and Howard Bell had some degree of resistance, so they were retested. Among varieties originated outside New Jersey, varying numbers of tests were made on Centennial, Champion, Early Black, and Shaw's Success.

The ratings for susceptibility to leafhopper feeding as shown by these tests are Woolman and Richards 18, Cherry Red and Garwood Bell 17, Howes 17.0 ± 0.16, Centennial 16.7 ± 0.6, Howard Bell 15.8 ± 0.64, Cham-

pion 15.7 ± 0.6, Taylor 15.4 ± 1.0, Early Black 13.4 ± 0.45, Plum 13.1 ± 0.9, McFarlin 13.0 ± (fixed), and Shaw's Success 9.5 ± 0.7. In this list the most susceptible (first four) varieties do not have standard errors as the ratings are based on single tests.

Although Champion and Howard Bell are significantly less susceptible than Howes, they are significantly more susceptible than McFarlin and have had no noticeable field resistance to false blossom. Early Black, which rates near McFarlin, has had marked field resistance. Plum, which rated very close to McFarlin in resistance, has been severely invaded by false blossom since the tests were made; however, it is planted in but one location and is not a homogeneous variety. Only one small block of Shaw's Success is known to the writer in New Jersey. An adjoining block of Garwood Bell of the same age was destroyed in 1940 because of heavy false blossom infection, but there was very little of the disease on Shaw's Success as late as 1942. The two tests of Shaw's Success were uniform and in agreement, and this variety merits attention because of its apparently high degree of resistance.

TABLE 2.—*Susceptibility of 143 cranberry seedlings to leafhopper feeding as indicated by the selective tests of 1940*

Parentage[a]	No.	Susceptibility of progeny										Means
		9	10	11	12	13	14	15	16	17	18–21	
Early Black self-pollinated	4	1		1		1	1					11.75
Early Black × Searls	6	1	1			3			1			12.33
McFarlin × Potter	6			1	1	3			1			13.00
McFarlin × Bennett	3				1	1		1				13.00
McFarlin × Early Black	49	1		5	10	12	11	4	3	2	1	13.25
McFarlin × Searls	11		1		2	2	3		2	1		13.73
McFarlin × Prolific	11		1	2		1	3	1	2	1		13.33
McFarlin self-pollinated	3					2			1			14.00
McFarlin × Stanley	2						1	1				14.50
Howes × Early Black	17				2	3	4	5	1	2		14.35
Howes × McFarlin	5					2	1		2			14.40
Howes × Searls	12					3	5		2	1	1	14.66
McFarlin × Berryberry	2						1		1			15.00
Howes × Potter	3							1		1	1	17.66
Total		3	3	9	16	33	30	13	16	8	3	13.74
McFarlin × sterile plants with symptoms resembling but distinct from those of false blossom	4			1	3							11.88
False-blossom plants, open-pollinated	5				2	2	1					12.85
LSD										(0.05)		1.69
LSD										(0.01)		2.24

[a] Reciprocal crosses are sometimes included for a given parentage.

Susceptibility of hybrid seedlings. The breeding work from which the 143 tested seedlings resulted was done in Wisconsin and there has been no opportunity for the writer to test the susceptibility of the parent varieties, except Early Black, Howes and McFarlin, which are also grown in New

Jersey. The seedlings are listed in table 2 according to parentage, with the frequencies of each group distributed on the scale of susceptibility. An analysis of these data resulted in a least significant difference of 1.69 at 5 per cent level and 2.24 at 1 per cent level between means of crosses. The McFarlin × Early Black seedlings are distributed fairly normally about a mean which is very close to average susceptibility rating of the parents. The Howes × Early Black seedlings show a mean susceptibility of 14.35 as compared with a value of 13.25 for the progeny of McFarlin × Early Black and 15.21 for the average of the two parents. The small number of progeny tested in the other crosses reduces the accuracy with which these means are estimated. This can be seen in the effect of the one very large value among the readings for the Howes × Potter seedlings. When all crosses involving Searls are considered, there is a suggestion that Searls would rate closer to Early Black and McFarlin than to Howes as a parent.

The comparisons above show a general tendency for varieties of known susceptibility to contribute their respective degrees of susceptibility to their progeny. The distributions of the progeny and the lack of unit dominance when resistant and susceptible varieties are crossed indicate that numerous factors are involved in the inheritance of resistance.

Additional tests were made by J. M. Delap, assisted by A. C. Borden, Jr., and W. C. Howard, in 1945 (6). All the available selections made on the

TABLE 3.—*Susceptibility of 351 cranberry seedlings to leafhopper feeding as indicated by 1945 tests*

Parentage	No.	Susceptibility of progeny											Means
		9	10	11	12	13	14	15	16	17	18	19	
McFarlin × Pride	7		2	2	2	1							11.4
Early Black × Searls	9		2	1	3	3							12.0
Shaw's Success × Paradise Meadow	6		1	1	2	1		1					12.4
Early Black × Aviator	6		1	2	1		1			1			12.5
Shaw's Success × Aviator	20	2		1	4	5	2	2	4				13.1
McFarlin × Shaw's Success	25			2	8	6	5		4				13.1
McFarlin × Prolific	9	1	1			2	3	1	1				13.1
McFarlin × Paradise M.	15			3	3	5		2	2				13.1
McFarlin × Early Black	52		3	2	12	13	9	4	8	1			13.3
Early Black × Shaw's Success	17			4	2	1	6	2	2				13.3
McFarlin × Searls	13			1	5	1	2	2	1	1			13.4
Aviator × Paradise Meadow	9			2	1		3	2	1				13.6
Centennial × Shaw's Success	20			3		4	6	5	2				13.8
McFarlin × Aviator	21			1		6	7	5	2				14.0
McFarlin × Centennial	32			1	3	5	9	11	3				14.0
Early Black × Centennial	9				2	2	1	1	2	1			14.1
Early Black × Prolific	5				1			4					14.2
McFarlin × Berryberry	13		1	1			1	6	3		1		14.6
Howes × Searls	21			1		4	3	6	2	4		1	14.9
Early Black × Howes	13				2		4	2	2	2	1		14.9
McFarlin × Howes	29			1	1	3	5	7	4	4	1	3	15.3
Total		3	11	29	52	62	67	63	43	14	3	4	13.7
LSD											(0.05)		1.18
LSD											(0.01)		1.56

basis of fruit and vine characteristics were included. Each test series included one cutting of each of 19 varieties, three of which were always Early Black, Howes, and Shaw's Success. McFarlin was included in a few of the series. Some selections were tested in two series, and the duplicate readings were in close agreement. Table 3 gives the ratings for 351 selections of the 21 crosses of the 13 parent varieties having at least five seedlings in the cross. Reciprocals are listed together. These data were statistically analyzed and significant differences were found between crosses. The least significant difference between means of two crosses was calculated to be 1.18 and 1.56 at the 5 per cent and 1 per cent levels, respectively. The seedlings of any one cross were not uniform in susceptibility but were distributed in a manner which approached normality when the number of progeny tested was large.

DISCUSSION OF PROCEDURES

Careful selection of uprights to be used in a selective test is a prerequisite for reliability of results, not only to obtain uniformity in the sample of each variety but to insure comparable vigor of the uprights of all varieties. Leafhoppers prefer to feed on highly vegetative shoots rather than on less rapidly growing uprights of the same variety. This was illustrated by the results of one series which included vigorous uprights of Howes and McFarlin from low spots of the bog where the water supply was ample, if not slightly excessive, and Howes from a higher site where both water and organic matter were somewhat deficient, but not to the extent of causing apparent pathological results. In this test the vigorous Howes rated 17.47, the less vegetative Howes 14.12, and vigorous McFarlin 13. In another test uprights in an advanced stage of false-blossom disease and low in vigor rated lower than healthy McFarlin. Uprights from a young, nonbearing bog should not be compared with those from fruiting vines of another variety. It should be remembered that "re-sanding" often restores an old, bearing bog to highly vegetative condition. In these tests fertile uprights of all varieties were usually used and the flowers or berries were removed when setting up an experiment; however, nonbearing uprights were employed in a few cases. No difference has been found in the uniformity of results with the two kinds of uprights, but both kinds should not be used in a single series. The leaf area of all uprights in a cage should be as nearly uniform as possible.

Since leafhoppers show a preference for the kinds of plants on which they formerly lived and fed, care is necessary to make sure that the leafhoppers used in a test are not collected from any of the cranberry varieties in the series. In the 1930–1932 tests notes were made of the bogs, but not always of the varieties, from which the insects were taken. Therefore, these results must be interpreted with discrimination and reserve. For example, the insects used in 1930 (7) were collected from a large bog which included Howes and four selected native Jersey varieties. The results suggest that the first collection was made from a "neutral" variety and that the second

included at least some insects from Howes. As a specific test of their food preferences, 1058 leafhoppers were collected from Howes cranberry and allowed to choose their food plant; 701, or 66 per cent, were counted on Howes and 34 per cent on *Chamaedaphne calyculata*. Of 988 leafhoppers collected from the latter, 588 or 60 per cent were counted on it, and only 40 per cent on the Howes. This shows that leafhopper vectors preferred to feed on the plants on which they had formerly lived. (The relative attractiveness of Howes and *Chamaedaphne calyculata* to neutral insects cannot be inferred from these results and a direct comparison with insects from a neutral species has not been possible.) Insects from *Chamaedaphne* were used in all series in 1940–1941. These vectors seem likely to be unbiased in their choice of cranberry varieties and also they are the chief source of infestation and reinfestation of cranberries in most localities.

Three uprights of each variety per cage were used in most tests up to the 1945 tests, but in a few cases the number was reduced to two. No difference was apparent in the uniformity of results.

In 1940 three different treatments of the cages were employed, which may be designated jarring, brushing, and turning. The relative efficiency of the methods was compared by four criteria. The dispersion of all insects in a series at each reading was determined, first on the basis of the seven cranberry varieties, then on the basis of the seven positions in a cage with regard to the direction of strongest illumination, using X^2 as an index of dispersion.[4] These are referred to as variety dispersion and light dispersion. High variety and low light dispersions were desired. The proportion of the insects found on the glass indicates the degree of disturbance or restlessness and should preferably be low. Finally, the most effective method of breaking up the continuity of numbers recorded in each position in consecutive readings and of overcoming the tendency of the vectors to remain in a single position, in order to induce successive variety choices, was measured by the correlation between consecutive readings. (1-r) was taken as an index of the independence of consecutive readings, indicating the number of insects that moved and made a new variety choice. A high index value was desirable.

Judged by every standard, the brushing method was the least efficient. It reduced the variety dispersion, increased the light dispersion, and increased the insects "on glass"; surprisingly, it did not materially increase the movement of insects from one position to another, apparently because the light stimulus remained constant.

Turning, the repeated 60-degree rotation of the cages, proved to be the best of the methods. Shifting of the varieties relative to the direction of the source of light supplied sufficient stimulus to increase the movement of insects, to raise the variety dispersion, to lower the light dispersion, and to

[4] $X^2 = \Sigma\ (x - \bar{x})^2/\bar{x}$, where x is the number of insects on a variety (or in one position) in a cage, $\bar{x}$ is the mean number per variety or position in all cages, and Σ indicates summation.

lower the percentage of insects on the glass. Where turning was used exclusively, a relatively large proportion of the insects was found on the glass the first 3 days, the light dispersion was high, the variety selection low, and there was little positional movement. With the fourth reading more of the insects were feeding, the cages had been turned 180 degrees, and many of the insects were seeking new positions. The first position of the insects after disturbance was determined largely on the basis of the direction of light; but as they became accustomed to their surroundings and the light stimulus was changed repeatedly, they made their successive choices of position more and more on the basis of the cranberry varieties, so that before the end of the experiment the influence of the varieties was greater than that of the light.

The proportion of insects on the glass was always high at the start of a series, then dropped and became fairly stable by the third or fourth reading, but had a tendency to rise toward the end of an experiment, due apparently to gradual drying out of the cuttings, even before any deterioration was visible to the observer.

In the 1930–1932 series the cages were placed so that sunlight did not fall directly on them at any time of day; but in 1940 the building used was so narrow that all cages were exposed to direct sunlight, slightly dispersed by passage through window screen, in early morning and late afternoon of a clear day, and it was found that neither the light dispersion nor the variety dispersion of readings made in direct sunlight differed appreciably from that of readings made in the shade or on a very cloudy day. The direction of strongest illumination played an important part in the distribution of the insects; but while the direction remained stable, the intensity of illumination had no apparent effect.

GENERAL DISCUSSION

Experience indicated the desirability of increasing the number of varieties per cage, increasing the number of cages per series, and using single uprights of each variety per cage so as to obtain a more accurate estimate of the error of sampling and at the same time to increase the ratio of "unknown" to standard varieties. This procedure proved effective in 1945. Restricted randomization of the varieties in all cages would eliminate any possible effect of the constant contiguity of varieties but would add somewhat to the labor of summation. Because of the relative inefficiency of the first three readings, it is suggested that the series be prepared but that no readings be taken until the fourth day. Six successive readings, taking the cage through one complete rotation, should be sufficient to complete the test, but if more readings are desired they can be continued until a rising proportion of insects on the glass indicates deterioration of the plant material.

During evaluation of these tests the question has been raised whether the insects would not feed and multiply normally on an unpalatable variety

if no more attractive forage was available, as in a large acreage planted to McFarlin. The attractiveness of the variety as determined in the test would seem to be unimportant in this case. The question cannot be answered categorically, but there is considerable indirect evidence bearing upon it.

Huber and Schwartze (5), working with red raspberries, compared the suitability of varieties and seedlings as food plants for the aphid vector, which reproduces viviparously and very rapidly under favorable conditions. They did not determine the food preferences of the insects by giving them a choice of varieties, but determined the reproductive capacity (or the degree to which this was inhibited) of the insect when fed on each variety 1 week after transferring a definite number of adult females. They found that on most varieties (which we may call class 1) the rate of reproduction was high; on some (class 2) it was low; on a few (class 3) there was no reproduction although the adults lived and appeared to feed normally; and on the Lloyd George variety even the adults were not able to survive (class 4). The authors grouped the varieties as susceptible, partly resistant, and resistant; but the fact that there were consistent differences in the rate of reproduction on varieties in class 1 and class 2 indicates a gradation from high to low susceptibility.

Among the plants tested by Blisard three ericaceous species are of interest. Insects fed and lived for more than a month on *Chamaedaphne calyculata* and *Leucothoe racemosa* (L.) Gray, but survived for only a short time on *Kalmia angustifolia*. It was subsequently found that wild plants of *Chamaedaphne* are the chief natural host of the leafhopper in New Jersey and Wisconsin and are usually infested with large numbers of the vectors. Obviously this plant is in class 1, in which the insects feed and reproduce normally. On the other hand, numerous attempts to collect *Scleroracus* from *Leucothoe* have failed, except for an occasional insect from a bush at the edge of the cranberry bog; thus this plant falls in class 3 in which insects feed but do not reproduce. *Kalmia*, of course, would fall in class 4.

To measure the relative rate of reproduction of leafhoppers on different varieties would be useful, but difficult since there is but one generation a year. Normal egg-laying and hatching have not been obtained in cages. Vector numbers vary widely between bogs of the same variety, and even between points a rod apart, due to local environmental conditions and management which affect the insects or the vigor of the host plant. Accordingly, there is no experimental evidence to show that the insects cannot feed indefinitely and perpetuate themselves from one generation to the next on any cranberry variety.

The measure of relative leafhopper density in common use by cranberry growers is the number collected in 50 sweeps with a standard net. Before the adoption of leafhopper controls, numbers as high as 700 per 50 sweeps were occasionally obtained in New Jersey on Howes, and 500 was common for Howes, Centennial, and other native varieties. These are unquestion-

ably class 1 food plants. Only once in the writer's memory or records did the number collected on Early Black exceed 200; usually the number was much less. Moreover, the milder control methods have been more effective on Early Black than on Howes, and reinfestation has taken place more slowly, so that many of the Early Black bogs in New Jersey do not receive annual treatments with insecticides; yet they are not heavily infested, and have not been seriously invaded by false blossom. It appears highly probable that the Early Black is a class 2 food plant. The plantings of McFarlin in New Jersey are neither numerous nor large enough to give a reliable comparison, but the fact that large acreages of this variety in Wisconsin have escaped severe damage from false blossom for many years lends support for placing McFarlin in class 2 also. The data on other cranberry varieties are fragmentary.

It thus appears highly probable that attractiveness to the vector is an expression of the suitability of a variety to maintain the vital functions of the insect, and that the rating of a variety on the scale of susceptibility, as determined by a selective test with neutral insects, is an indirect measure of the ability of the insect to live and multiply on that variety, whether or not other more attractive varieties are available.

In Early Black and McFarlin, with S-ratings of about 13, false-blossom disease has spread less rapidly than in Howes, which has an S rating of 17. The difference in rate of spread has been much greater than the ratio between their respective S ratings would indicate. If, in the vicinity of 13 on the scale, varieties are found on which the insect reproduction is inhibited to such a degree that populations of dangerous density do not develop, there will be a marked drop in the spread of the disease and the apparent discrepancy will be explained. Further light should be thrown on this by the behavior of the insects on Shaw's Success variety (S = 9.5).

The problem of hybridizing varieties of cranberries resistant to false blossom is complicated by the possibility of the existence of leafhopper strains that are adapted to life on a resistant variety. The first suggestion that such strains might occur came from Blisard's test on *Leiophyllum buxifolium* (Berg.) Ell. and three species of *Gaylussacia* (Table 1). Most of the insects lived only 3 or 4 days, the same as those deprived of all food; but 10 to 20 per cent were able to feed and lived for more than a month, or until the end of the experiment. These plants are common in the New Jersey cranberry area and at least three species occur in fairly large colonies or masses. The fact that infestations of leafhoppers have not been found on them indicates that the plants are probably on the borderline between class 3 and class 4, and that the insects cannot perpetuate themselves even though a few adults were sufficiently adaptable to live to a normal age. The possibility is recognized, however, that a slightly greater degree of adaptability might enable a few insects to reproduce normally on a generally unsuitable species and thus develop a specialized strain adjusted to and actually preferring a plant formerly considered unsuitable.

Strains with diverse feeding preferences actually occur, but it is not known whether the perpetuative capacity of the strains differ. Other selective tests have given indications of vector adaptation to cranberry varieties and there is good reason to believe that this process has actually taken place on cranberry bogs. This would be the simplest explanation of the instance where large numbers of the vector were found on a large bog of Early Black, of invasion of the Plum variety by false blossom, and of observed cases in which a variety has been susceptible to the spread of the disease in one locality and apparently resistant in another equally exposed to infection. When a variety has remained free from infection over a large area for several years and then has been invaded, the cause may have been either (1) a lack of exposure to viruliferous insects during the earlier years or (2) the appearance of a vector strain adapted to the variety, or both. A number of specialized vector strains arising on a single partially resistant variety need not necessarily be genetically related; but the frequency of their origin, and hence the rate of development, would presumably depend upon the proportion of native vectors that were able to perpetuate themselves and upon the number of insects from which the selection was made. Thus, the likelihood of specific strains arising and rapidly increasing would be greater on a class 1 than on a class 2 or class 3 plant.

The selective test can only indicate the resistance to native or neutral vectors, and the probability of the existence of specialized strains. Once such strains develop, it can give no clue to the probable rate of spread of false blossom on a variety if the adapted insects are not controlled. However, effective control measures have been found and are being extensively used. Any variety in classes 1, 2, and 3, planted in any location suitable for cranberries east of the Rocky Mountains, will be subject to infestation and annual reinfestation from wild plants or from other varieties of cranberries. The physiological suitability of a variety as a food plant for the vector is, therefore, likely to exert a continuing influence on the rate of spread of false blossom in the variety.

SUMMARY

Thirteen named cranberry varieties and 494 seedlings of known parentage were used in selective tests in which the leafhopper vector (*Scleroracus vaccinii*) of the false-blossom virus was given a choice of varieties on which to feed, in order to estimate the relative attractiveness of the varieties as food plants, and hence their liability to contract the disease. The results indicate that in hybridization, varieties of known susceptibility to vector attack tend to contribute their respective susceptibility to the progeny. Neither susceptibility nor resistance suggests unit dominance; they seem to be the product of numerous factors. The tests indicate that one named variety (Shaw's Success) and several hybrid seedlings are more resistant to vector attack than McFarlin which possesses considerable resistance in plantings. No cranberry variety was entirely immune from vector feeding.

The test was evaluated as an indicator of resistance to vector attack. It is concluded that attractiveness, an expression of the suitability of the variety as a source of food for the insect, depends on the ability of the insect to survive and increase from year to year. Therefore, the rating test is an indirect measure of the probable degree of survival and rate of reproduction of the vector, regardless of whether other more palatable varieties are present. The advantages, disadvantages, and limitations of the test are noted. The evidence indicates that it is entirely feasible to originate varieties from parental material now on hand which will have considerably more resistance to vector attack than any variety now grown.

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LEAF RUST OF WHEAT, PUCCINIA RUBIGOVERA TRITICI, IN URUGUAY[1]

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(Accepted for publication March 26, 1951)

INTRODUCTION

According to the information contained in Uruguay's *Plan Agropecuario Nacional* (9), wheat is the most important crop in that country's agricultural economy. It occupies an area of over a million acres and is grown widely in the southwestern and western provinces. More than three-fourths of the wheat acreage (77.5 per cent) is located in the following six provinces: Soriano (25.0 per cent), Colonia (16.3 per cent), Rio Negro (12.5 per cent), Paysandú (10.0 per cent), San José (7.5 per cent), and Canelones (6.2 per cent).

Several cereal rust surveys have been made in Uruguay, the earliest by Gassner (3) in 1907. As in other parts of the world, wheat in Uruguay is often severely damaged by recurrent cereal rust epidemics. Chester (1) cites Gassner as the authority that the orange leaf rust, *Puccinia rubigovera tritici* (Erikss. & Henn.) Carl. (*P. triticina* Erikss.), causes by far the greatest damage to the wheat of Uruguay.

To the writers' best knowledge, no detailed research has been reported to date on Uruguayan leaf rust, either within or outside the borders of that country. Casual reference is made by Favret (2) to unpublished studies of Vallega dealing to some extent with the occurrence of race 15 in Uruguay.

A better knowledge of the occurrence and distribution of leaf rust races in Uruguay and of the relative resistance of wheat varieties and selections to such races is highly desirable, if the injurious effects of leaf rust are to be reduced.

There is an appreciable amount of published information available on the occurrence of physiologic races of orange leaf rust in Uruguay's neighboring countries, Brazil, Argentina, and Chile. Vallega (6) reported the

[1] Joint contribution from the Regional Agronomic Service, Directorate of Agronomy, Ministry of Ganaderia and Agriculture, Republic of Uruguay, and from the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, in cooperation with the Division of Plant Pathology and Botany, Department of Agriculture, University of Minnesota. Published as paper No. 2280 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station. This paper also will be published in Spanish in Archivo Fitotécnico del Uruguay. Grateful acknowledgment is offered to Dr. Luis Alberto Brause and Ing. Agr. Agustin Trujillo Peluffo of the Ministry of Ganaderia and Agriculture in the Republic of Uruguay; to Dr. K. S. Quisenberry and Dr. H. A. Rodenhiser of the United States Department of Agriculture; and to Dr. E. C. Stakman and Dr. Helen Hart of the University of Minnesota for their interest in the initiation and prosecution of this study.

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isolation of races 19, 64, and 105 from Brazilian collections. Da Silva (5) several years later isolated races 10, 15, 26, 31, 49, 122, and 129 from uredial material obtained in Brazil. In Argentina, Vallega (8) found races 5, 13, 20, 26, 49, 57, 62, 105, and 114 to be more or less common. From leaf rust specimens collected in Chile, Vallega (7) isolated races 15, 68, and 114. Vallega (7, 8) studied the seedling reaction of many varieties and selections of wheat to the three Chilean and to six of the nine Argentine races referred to above. Very few of the wheats tested were resistant to all of the races.

Physiologic specialization studies of Latin American collections of leaf rust have been made from time to time by graduate students at the Cereal Rust Laboratory on the St. Paul Campus of the University of Minnesota. The first exploratory studies of Uruguayan collections,[4] obtained in 1944, were carried out under the guidance of the junior author[5] and constitute a part of this paper.

MATERIALS AND METHODS

The first uredial material studied was collected during 1944 in only two of Uruguay's provinces, Colonia and Salto. The material for the more recent study had been collected in the fall and winter of 1949 in the following five provinces: Colonia, Montevideo, Paysandú, Salto, and Soriano.[6] Unfortunately, all of the Soriano specimens had died by the time they reached the Minnesota laboratory. The majority of the other specimens produced infection to a very satisfactory degree: 63.6 per cent in 1944 and 90.7 per cent in 1949, in relation to the number of specimens involved in each case. Seed of the differential hosts, used in the identification of the physiologic races isolated from the various specimens, and of the varieties and selections, tested for their comparative seedling reaction, were received from several sources.[7] The identity of the unipustular isolates was established by means of the diagnostic key published by Johnston et al. (4) in 1942.

Physiologic Races

It will be noted from the data presented in table 1 that the orange leaf rust in Uruguay consists of many physiologic races. The 31 isolates ob-

[4] Thanks are due to Dr. G. J. Fischer and Ing. Agr. Rubens Ribeiro of the National Phytotechnical Institute at La Estanzuela for wheat leaf rust specimens, from the provinces of Colonia and Salto, originally sent to Mr. da Silva.

[5] Mr. Ady Raul da Silva of the Experiment Station at Curitiba, Brazil, and a graduate student at the University of Minnesota, rendered helpful assistance during the initial stages of the investigation, transmitting rust specimens and cooperating in the racial identification of isolates.

[6] The writers are indebted to Messrs. G. J. Fischer and Rubens Ribeiro of the National Phytotechnical Institute at La Estanzuela, to Messrs. Jorge W. Diaz, Arturo Montoro Guarch, and Omar Odriozabal of the Agronomic Faculty of the University of Montevideo, and to Mr. Raul Manfredi of the Ministry of Agriculture for the generous supply of uredial material on different wheats.

[7] Sincere thanks are expressed to the following gentlemen for the liberal supply of seed: D. G. Fletcher of the Rust Prevention Association in Minneapolis, Carlos Barbosa Gomez of the Mexican Ministry of Agriculture, A. M. Guarch, C. O. Johnston of the U. S. Department of Agriculture, Rubens Ribeiro, and W. L. Waterhouse of the University of Sydney in New South Wales, Australia.

tained from the 1944 collections yielded 19 different races, whereas the 62 isolates from the 1949 collections comprised 18 races. While a total of 27 races was identified, only 10 of these were present both in 1944 and 1949. These races were: 5, 10, 31, 35, 37, 52, 61, 86, 99, and 105. Races 2, 6, 47, 49, 53, 77, 93, 117, and 128 were isolated from the 1944 collections only, and races 1, 8, 9, 13, 15, 20, 43, and 50 were identified only in the 1949 collections. The occurrence of race 15 in Uruguay had been noted earlier in Vallega's unpublished data. The authors thus far have not encountered

TABLE 1.—*Frequency distribution of the physiologic races of orange leaf rust, Puccinia rubigovera tritici, isolated from collections obtained in Uruguay during the fall and winter of 1944 and 1949, respectively*

Identity of races isolated	Geographic distribution of physiologic races identified							
	1944			1949				
	Colonia	Salto	Number of isolates	Colonia	Monte-video	Pay-sandú	Salto	Number of isolates
1	....	....	....	....	1	....	....	1
2	....	2	2	....	....	....	....	....
5	....	1	1	1	....	....	....	1
6	2	....	2	....	....	....	....	....
8	....	....	....	....	4	....	....	4
9	....	....	....	1	....	....	2	3
10	....	2	2	....	5	1	1	7
13	....	....	....	....	1	2	....	3
15	....	....	....	....	1	....	....	1
20	....	....	....	....	2	3	1	6
31	1	1	2	1	1	....	5	7
35	1	....	1	....	1	....	1	2
37	1	....	1	2	....	2	....	4
43	....	....	....	....	....	1	....	1
47	2	....	2	....	....	....	....	....
49	....	1	1	....	....	....	....	....
50	....	....	....	2	3	2	....	7
52	2	1	3	4	....	....	....	4
53	....	1	1	....	....	....	....	....
61	1	....	1	....	1	....	1	2
77	2	1	3	....	....	....	....	....
86	2	....	2	....	4	1	....	5
93	1	....	1	....	....	....	....	....
99	1	....	1	....	....	....	1	1
105	....	1	1	....	3	....	....	3
117	1	....	1	....	....	....	....	....
128	3	....	3	....	....	....	....	....
Total number of isolates	20	11	31	11	27	12	12	62
Total number of races	13	9	19	6	12	7	7	18

races 19, 64, 122, and 129 reported from Brazil, races 57 and 62 found in Argentina, or race 68 occurring in Chile, among Uruguayan collections. Likewise they did not find race 26 which is common to Brazil and Argentina or race 114 which is common to Argentina and Chile. It must be borne in mind, however, that the present study was limited in scope and is

to be considered as preliminary in nature. With the exception of races 8, 47, 53, 86, 99, and 117, leaf rust races very much akin to, though not necessarily identical with, the other races isolated from the Uruguayan collections have at one time or another been found also in the Upper Midwest area of the United States.

Varietal Tests

After the racial identification of the unipustular cultures had been completed, pure strains of ten Uruguayan and five North American races were selected for studying the seedling reaction of certain varieties, hybrids, and selections in the greenhouse. The North American races were isolated from leaf rust specimens collected in the Upper Midwest region of the United States, embracing the States of Minnesota, North Dakota, South Dakota, and Montana. The results obtained are in table 2. Only one variety, Cappelli (C. I. 12452), was very resistant to each of the Uruguayan and United States races involved in the test. Two other varieties, Gabo (C. I. 12795) and Lee (C. I. 12488), approached that record except that both were variably mesothetic to the Uruguayan race 15, instead of being highly resistant to it. To this extent, these two varieties demonstrated the difference between the pathogenic behavior of the Uruguayan race 15 and that of the United States race 15. Biotypes among races 5, 13, 20, 31, and 86 have been demonstrated on the Australian differential variety, Thew (C. I. 5002), which is thoroughly susceptible to the United States isolates of these races, but highly resistant to their Uruguayan counterparts. These and similar differences in other racial biotypes must constantly be borne in mind when the significance of the infection record of any physiologic race is evaluated in its relation to plant pathologic or plant genetic problems. This study has revealed that Aniversario (C. I. 12578) differs markedly from Klein Aniversario in seedling reaction to most of the Uruguayan races tested. It also shows that the seedling reaction of Frontana to several Uruguayan races is appreciably different from that of Fronton. These two varieties, however, are practically identical in their seedling reaction to the five United States races under review. Klein Titan (C. I. 12615) has thus far been extremely resistant in the seedling stage to all Upper Midwest races tested; Rio Negro (C. I. 12469) has been resistant to most of them. Both varieties reacted less favorably to the Uruguayan races.

SUMMARY AND CONCLUSIONS

A preliminary study has revealed the existence of a greater variety and complexity of physiologic races of the orange leaf rust of wheat in the Republic of Uruguay than has heretofore been reported for the neighboring countries, Brazil, Argentina, and Chile. Uruguayan wheat is subject to attack of as many as 27 different physiologic races of leaf rust and probably even more. Races 10 and 31 were most frequent and widely distributed in Uruguay; each constituted 9.7 per cent of the total number of isolates iden-

TABLE 2.—*Comparative seedling reaction of certain varieties and selections of wheat to several physiologic races of Puccinia rubigovera tritici of importance in the Republic of Uruguay and in the Upper Midwest area of the United States*

Material tested		Types of infection produced by specified rust races													
		Republic of Uruguay										United States			
Varietal name or symbol	C.I. No.	9	10	13	15	20	31	37	43	50	86	5	9	15	52	126
Aniversario	12578	4–	3++	4–	1±	4–	3+	4–	4	4–	1±	1±	1+	1++	1+	1–
Brevit	3778	2–	3++	2=	1+	4	0;	1++	3+	4	3++	0;	1+	1+	1=	2
Cappelli	12452	0;	1	0;	1++	1+	1–	1	1–	1=	1–	0;	1–	1	1	0;
Carina	3756	1++	4–	3++	0;	4±	1+	2	4	2=	4	1=	2±	1+	0;	1
Democrat	3384	1–	0;	1–	4	0;	1	2=	1++	0;	2=	3++	1=	3++	4–	3++
Frontana	12470	1++	0;	1+	0;	X–	1–	0;	X+	X±	0;	0;	1±	0;	1±	1–
Fronton		X–	3++	X++	1=	3+	X	X–	X++	X±	1+	1=	1	0;	1	1=
Gabo	12795	1–	1=	0;	X+	1=	0;	1=	1–	1	0;	1–	1	1±	0;	1±
Hussar	4843	2–	1++	4	0;	4	3++	2	1+	2–	1±	2–	2+	1	3+	2
Hybrid DP1(31)1		3++	4–	3++	0;	3+	4–	3++	3++	4–	0;					
Hybrid DP1(31)2		3+	3++	4	0;	3++	3++	3+	4–	4–	0;					
Hybrid DP1(31)3		3++	4–	4±	0;	4–	4	4±	3++	4	0;					
Hybrid DP1(31)001		3++	3++	4	0;	3++		4	4–	4–	0;					
Hybrid DP1(36)032		4–	4	4	X±	4	4+	4–	3++	4+	2=					
Hybrid DP1(64)03		4–	4–	3+	X–	3+	X++	3++	4–	4	0;					
Hybrid DQ3(150)2		X+	X+	4–	X	4–	3+	X+	4	4–	3++					
Hybrid EG127		4–	4–	4	X±	4+	4++	4	4	4	X±					
Hybrid EL115		4±	X+	4++	1=	4	X++	X+	3++	X++	1–					
Klein Aniversario		1+	1+	1±	X–	X	1+	1	1±	1–	1					
Klein Lucero		1	X±	1=	X+	0;	1=	X+	3+	1±	1–					
Klein Titan	12615	1	3+	1–	X–	1±	0;	X±	X=	1	1±	1±	0;	1+	0;	1–
Klein 157	12586		X±	4	3+	4	4	4–	4	4	4	3+	1+	3+	1++	3±
Lee	12488	1	1=	0;	X++	0;	0;	1=	1	0;	1=	0;	0;	0;	1=	1–
Litoral Precoz		4	3++	4–	3++	4+	3++	3++	4+	3++	1+	4+	4–	3++	X±	3++
Loros	3779	4++	4	3++	1–	3++	4	4±	3++	4	3++	1=	3++	1+	1	4
Malakof	4898	4–	3++	4	0;	4	3++	4	4–	3++	1	4–	3+	0;	3++	4±
Marquis	3641											4	4	3++	4–	3++
Mediterranean	3332	0;	1–	0;	4	0;	1+	2–	0;	1±	4–	3++	1±	3++	4	4–
Petiblanco		4	4–	4+	X=	4+	4–	3++	4	4–	1+	X±	4	0;	1=	X–
Petirojo		4±	4++	4++	1	4	4+	3+	4+	X++	1±	X–	3++	X=	X–	1++
Quanah	12145	4	4	4+	4	4–	4	X+	X+	4	3++	3++	4+	4	3++	3+
Rio Negro	12469	X++	2=	3++	0;	3+	4++	3++	3+	3+	0;	0;	3+	0;	0;	3+
Selection 7d		0;	1–	0;	3+	0;	1=	1+	1=	1±	1=					
Selection 26n		2+	3+	X+	4–	X++	4	4	3++	4	4–					
Selection 33e		X±	X++	X	X–	X–	X=	X+	X++	X+	4–					
Selection 44d		3++	4–	4	3++	4	4+	4+	4–	4	3++					
Sinvalocho	12595	0;	1	0;	3+	0;	0;	0;	1=	0;	1±	4+	0;	4	3+	3
Thatcher	10003	4–	3++	4	4	3++	4	4–	3++	4=	4	3++	4	3++	4	3++
Thew	5002	4+	4	1++	4–	1+	0;	3+	3++	4–	1++	4	3++	4–	4±	4+
Webster	3780	4	4++	4–	0;	4	3++	1++	2=	1+	4	1=	4–	0;	1	1++

tified and each was endemic to three of the four provinces from which viable uredial material had been obtained. Next in frequency were races 50, 52, and 86; each constituted 7.5 per cent of the total number of identified isolates, but were less widely distributed. Somewhat less frequent were races 20 (6.5 per cent) and 37 (5.4 per cent).

Physiologic races bearing the same numerical designation often are not identical genetic entities. Additional differential varieties or selections may bring out sharp departures in their infection pattern. Thew is an excellent example of a rarely used differential variety that is capable of screening out biotypes or even distinguishing physiologic races. Gabo and Lee are effective in screening out biotypes of races 11 and 15. Both of the latter varieties are very desirable wheats agronomically in their native habitats and might prove of value also in Uruguay, either as commercial or parental material. The same is largely true of Cappelli which possesses extraordinarily high seedling resistance to all Upper Midwest races tested to date, 29 races in all. However, it has occasionally been severely affected by leaf rust in Mexico.

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THE OCCURRENCE OF PHYTOPHTHORA CINNAMOMI IN THE SOIL UNDER PINE STANDS IN THE SOUTHEAST

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(Accepted for publication March 29, 1951)

Phytophthora cinnamomi Rands was first described in 1922 by Rands (8) as the cause of streak cankers of *Cinnamomum burmanni* Blume in Sumatra. White (10) found it causing root rot of *Rhododendron* in New Jersey and his report in 1930 was the first record of the fungus in the United States. In 1945 Crandall, Gravatt, and Ryan (6) described a root disease of *Castanea* spp. and of various kinds of nursery stock and demonstrated that it was caused by *P. cinnamomi*. They isolated the fungus from chestnuts and chinkapins in Arkansas, Mississippi, Tennessee, Alabama, Georgia, North Carolina, and South Carolina, and submitted evidence to show that root rot caused by *P. cinnamomi* was probably responsible for the recession of the chestnut in the Southeast long before blight eliminated that species as a timber tree in the East. In recent years pathologists in various parts of the country have reported *P. cinnamomi* causing root disease of several woody hosts, usually under conditions of excessive soil moisture (9, 11).

In 1948 *P. cinnamomi* was isolated from the dead small roots of shortleaf pine (*Pinus echinata* Mill.) affected by littleleaf disease (1). This discovery was of particular interest because Copeland (5) had found that the disease was most severe on South Carolina soils having poor internal drainage. Furthermore, the distribution of littleleaf disease (7) coincides markedly with the reported distribution of chestnut root rot, which has been attributed to *P. cinnamomi*. In several counties in Georgia where shortleaf pine stands are badly affected by littleleaf, the dying of chestnuts in the 20 years prior to 1875 was spectacular enough to warrant mention in a report on forest statistics published in 1875, as cited by Clinton (4).

Unfortunately *P. cinnamomi* proved difficult to isolate from the roots of littleleaf-diseased pine. Copeland[2] has found that approximately one-third of the roots of littleleaf-diseased trees are in a necrotic condition. However, most of these necrotic roots were in advanced stages of decay and usually only saprophytic organisms could be isolated from them. *P. cinnamomi* was isolated from only 2 per cent of the roots from which isolations were attempted from 11 trees, utilizing as far as possible only small roots that appeared to be recently killed. To get any appreciable number of suitable roots for isolation purposes it was necessary to do a great deal of digging.

Later it was discovered that *P. cinnamomi* could be isolated directly from the soil by using apples as the differential medium (2). In using this method, 24 small soil samples were usually taken in an area to be tested. Each sample was taken with a trowel or soil tube to a depth of 6 in. In

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[2] Copeland, O. L. A study of sizes, condition, and distribution of shortleaf pine roots in five Piedmont soils. Unpublished report. 1948.

the laboratory two portions of each sample were packed into two ½-in.-diameter holes bored almost, but not quite, through an apple. The soil in the apple was moistened with distilled water, the holes were covered with Scotch tape, and the inoculated apples were incubated at room temperature. *P. cinnamomi*, if it invaded the tissues, caused a firm rot of the apple in from 5 to 10 days. Confirmation of the rot as being caused by the fungus was made by plating bits of the rot on cornmeal agar.

With this apple technique, a study was made of the relative abundance of *P. cinnamomi* in the soil around littleleaf and healthy trees in littleleaf stands near Athens, Georgia, and around healthy trees in littleleaf-free stands (3). Approximately 42 per cent of the soil samples taken under littleleaf trees contained the fungus. By contrast, *P. cinnamomi* was isolated from 15 per cent of the samples taken under near-by healthy trees and from only 5 per cent of the samples from under healthy trees in healthy stands. A similar study made by Toole[3] in a littleleaf stand near Asheville, North Carolina, and in surrounding healthy stands, with watermelons as the differential medium, showed about the same relationship.

TABLE 1.—*Distribution of plots according to States and types of pine stands where samples were taken*

State	Number of plots	Type of pine stand				
		Shortleaf	Shortleaf-loblolly	Loblolly	Slash-longleaf	Mixed hardwood-pine
Alabama	12	1	3	4	4	.....
Florida	3	.....	.....	.....	3	.....
Georgia	26	1	7	15	.....	3
Mississippi	2	.....	1	1	.....	.....
North Carolina	1	.....	.....	.....	.....	1
South Carolina	13	.....	.....	13	.....	.....
Texas	4	4	.....	.....	.....	.....
Total	61	6	11	33	7	4

Later in 1949 and 1950, soil samples were assayed for *P. cinnamomi* from 153 plots from shortleaf pine stands in the area generally affected by littleleaf in South Carolina. These samples were from ¼-acre plots established in 17 Piedmont counties by O. L. Copeland and were not taken in relation to the location of any particular trees. Examination of the data from this study showed no correlation between the abundance of *Phytophthora* in the soil, as determined by 24 samples per plot, and the condition of the stands in respect to littleleaf. This lack of correlation between the relative amount of *P. cinnamomi* in the soil and stand condition raised the question as to the distribution of the fungus in pine stands outside the littleleaf area. A survey of such pine stands was made during the winter and spring of 1950. In this survey 24 soil samples were taken from each plot, which was approximately ⅛ acre in area, and located without reference to individual trees.

As shown in table 1, 61 plots were selected in five different forest types

[3] Toole, E. R. Unpublished data. 1949.

in seven States. Stands of loblolly pine (*Pinus taeda* L.) were the most numerous and represented the common forest type in much of the area in which the plots were located. Pure shortleaf was represented by only six plots and shortleaf-loblolly by 11. Only seven plots were located where slash pine (*P. caribaea* Morelet) and longleaf pine (*P. palustris* Mill.) predominated. The four mixed hardwood-pine plots were located in the mountains in North Georgia and western North Carolina, beyond the general range of littleleaf. Wherever possible plots were placed in stands having shortleaf, either pure or in mixture, but such stands were difficult to find outside the littleleaf area. Loblolly stands were next in order of preference. Plots were located in the other types when shortleaf or loblolly could not be found. Stands containing shortleaf and loblolly pines were preferred because these species are the ones commonly found in littleleaf areas, and a comparison of the relative abundance of *P. cinnamomi* in the soil under these species in littleleaf stands and non-littleleaf stands outside the general range of the disease was one object of the study.

Figure 1 shows the distribution of the plots in relation to the known

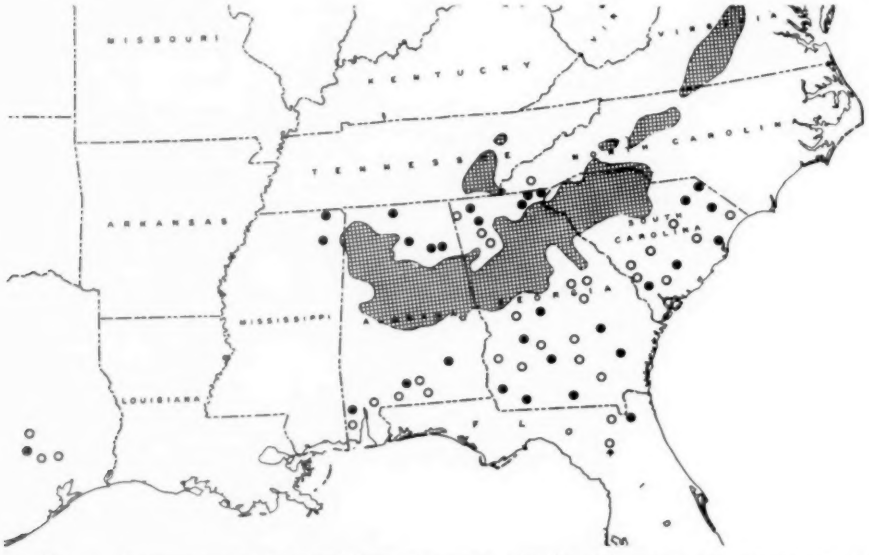

FIG. 1. Occurrence of *Phytophthora cinnamomi* in the soil under pine stands outside of the range of littleleaf disease. Hatched areas indicate distribution of littleleaf on shortleaf pine. Circles give locations of plots not yielding the fungus; black dots, plots yielding *P. cinnamomi*.

distribution of littleleaf. In the areas south and east of the littleleaf belt in Alabama, Georgia, and South Carolina, *P. cinnamomi* was isolated from soil samples from approximately half of the plots. This demonstrates the wide general distribution of the fungus in the Southeast. In these stands it apparently exists in the soil without evident injury to the pines.

The occurrence of *P. cinnamomi* in the soil under different types of pine stands outside the littleleaf area was determined for 61 plots. The fungus

was isolated from 52 per cent of the 50 plots in stands having shortleaf or loblolly pines, either pure or in mixture. In the South Carolina study already referred to the fungus was found in the soil of 139 of the 154, or 91 per cent of the plots. These plots were in shortleaf or in mixtures of shortleaf and loblolly. *P. cinnamomi* appears to be much more prevalent in the soil under pine stands in the Piedmont than under pines of the same species in the Coastal Plain outside the range of littleleaf. The fungus was found in soil samples from only one (14 per cent) of seven plots of longleaf pine or slash pine. Although 75 per cent of the plots (three of four) in the mixed hardwoods-pine had *P. cinnamomi* in the soil little significance can be attached to this figure in view of the small number of plots.

Some data on the relative abundance of *P. cinnamomi* in the soil in each plot are in table 2. The fungus was not isolated from the soil from 31 of

TABLE 2.—*Relative abundance of P. cinnamomi in the soil under different types of pine stands outside the littleleaf area*

Number of plots from which isolation was attempted	Number of soil[a] samples per plot yielding *P. cinnamomi*
31	0
17	1 to 3
6	4 to 6
3	7 to 9
4	10 to 12
0	13 or more

[a] Twenty-four samples were taken from each plot.

the 61 plots outside the littleleaf area. The range of frequency with which it was isolated from the other 30 plots varied from 1 to 12. Half of the 30 plots from which *Phytophthora* was isolated had the fungus in only one or two of the 24 samples taken from each plot. Eight, or 26 per cent, of the 30 plots yielding *P. cinnamomi* gave recoveries of the fungus from six or more of the 24 samples. The relative abundance of the fungus in these plots was much lower than for the plots in the littleleaf area of South Carolina where the isolations ranged from 1 to 17 per plot for the 139 plots in which *P. cinnamomi* was present. Only 17 per cent of the 139 South Carolina plots yielded one or two isolations from 24 samples, while 50 per cent of the plots had the fungus in six or more of the 24 soil samples taken from each plot.

Soil type and condition were probably important in the low incidence of the fungus in the Coastal Plain where it was usually present in only small amounts in plots having sandy soils. The exceptions to this occurred in several plots having a high humus content along with the sandy soil.

In summary, the development of a method of detecting *Phytophthora cinnamomi* in the soil regardless of its relationship to a diseased host made it possible to obtain information on its distribution in the soil under pine stands in the Southeast. This information was considered desirable because

the fungus has been found associated with littleleaf-diseased shortleaf pine and is more abundant in the soil under diseased than under healthy trees. Furthermore, soil assays for *P. cinnamomi* in connection with a study of the relation of soil characteristics to the presence of littleleaf in South Carolina disclosed that the fungus was present in 91 per cent of the plots from which samples were taken. There appeared, however, to be no correlation between the relative amount of *P. cinnamomi* in these plots and the severity of littleleaf. When the study was extended to pine stands outside the littleleaf area the fungus was found in the soil under 48 per cent of all stands in which plots were located and under 52 per cent of these stands having shortleaf and loblolly pine, the species also present on plots in the littleleaf area. *P. cinnamomi* was found to occur in practically all areas tested in the Southeast and in places remote from any known connection with a recognized disease.

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A NEW TECHNIQUE FOR INCUBATING SEED CORN IN COLD SOIL FOR DISEASE TESTS

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(Accepted for publication March 27, 1951)

Extensive germination tests with seed corn in cold soils may be made without special equipment other than an ordinary electrically controlled refrigerator and adequate space in a well-lighted room or greenhouse in which to complete a warm-temperature phase of the test.

The kernels are mixed with soil in a glass tumbler or other suitable container so that every kernel is in contact with soil. An ordinary 250-cc. tumbler will hold 200 average-size kernels. Water is added until the soil is uniformly wet but not saturated and the container, covered with waxed paper, is placed in the refrigerator for incubation. The time and the temperature of incubation vary with the experiment. Following incubation, the kernels are removed from the tumbler and planted in soil in a warm room or greenhouse. Data are taken on seedlings in the 3- to 4-leaf stage.

The advantage of this glass-tumbler method is that large and expensive refrigeration equipment is not needed. Its adequacy for the study of both practical and fundamental aspects of the corn seedling disease problem is demonstrated in experiments reported here.

EVALUATION OF DUST PROTECTANTS

The glass-tumbler technique was compared with the method used formerly in a large walk-in refrigerator for testing seed-treatment dusts. Three dust fungicides were tested by the two methods: Experimental Fungicide No. 640 (zinc copper chromate) and Experimental Fungicide No. 224 (zinc mercury chromate), both from Carbide and Carbon Chemicals Corporation, and Arasan (tetramethylthiuram disulfide 50 per cent) from E. I. duPont de Nemours and Company. The dusts were applied in excess to graded kernels ("medium flats") of a Wisconsin experimental 3-way corn hybrid. The kernels had mechanically inflicted crown wounds, but otherwise were physically sound and free from seed infections. The nature of the seed and the test conditions insured abundant disease in the untreated controls with a minimum of variability in results caused by extraneous factors.

A peat soil, used in all routine low-temperature tests with corn at the Wisconsin Agricultural Experiment Station, and known to be infested with highly pathogenic strains of *Pythium debaryanum* Hesse and *P. ultimum* Trow, was used in this experiment.

For the test in the large walk-in refrigerator, the kernels were planted

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in 5-in. flower pots, in four 25-kernel replicates for each of the dust treatments and the untreated control, with a vermiculite mulch to prevent drying out during the low-temperature incubation. After 14 days at 11° C. the pots were moved to the greenhouse where the air temperature fluctuated between 18° and 24° C.

In the glass-tumbler test, 100 kernels from each dust treatment and the untreated control, embedded in peat soil in the tumblers, were incubated in the refrigerator and then planted in 25-kernel replicates, in peat soil in flower pots, in the warm greenhouse.

Similar results were obtained from the two methods of testing (Fig. 1).

FIG. 1. Comparison of methods for testing dust disinfectants used in corn seed treatment. Results when glass-tumbler technique was used (front row of pots) were almost identical with those obtained by the large-refrigerator method (rear row) when the indicated treatments were tested by both methods.

Pre-emergence death (primarily seed decay) was nearly 100 per cent for untreated seed in both methods of testing. Similarly, the numbers of healthy seedlings following the dust treatments were almost identical for the two methods of testing.

EFFECT OF INCUBATION TIME AND ENVIRONMENT ON DISEASE

The favorable results obtained with the glass-tumbler technique in the seed-treatment experiment suggested the use of this method in a study of the cold soil germination problem in corn.

Arasan-treated and untreated seed like that used in the seed-treatment experiment was incubated at 4° and at 11° C. for 5, 10, and 15 days, in both peat soil and clean fungus-free sand. The incubations were timed so that all seeds were removed from the refrigerators on the same date, for the replanting in the warm greenhouse. Treated and untreated kernels were planted in the greenhouse in peat soil and in clean sand as nonincubated controls.

Six 25-kernel replicates for each treatment were planted in the greenhouse in a layer of clean sand over compost soil in 5-in. flower pots. This avoided disease effects (mainly stunting) which occur even at favorable temperatures when crown-wounded corn kernels are planted in *Pythium*-infested soils.[2] The pots were mulched with vermiculite. The data in table 1 show no evidence of any appreciable amount of disease or of deleterious effects when either treated or untreated kernels had been incubated in the fungus-free sand. Excellent stands of healthy seedlings were obtained even when untreated seed had been incubated 15 days at 4° C. This indicates that low temperature, in itself, was not injurious to the crown-wounded seed under the conditions of the experiment.

Incubation in peat soil resulted in a high incidence of seed decay in unprotected seed. Seed decay reached 100 per cent in the 10- and 15-day incubations, both at 4° and 11° C. Except for the 15-day incubations at 11° C., Arasan seed treatment afforded nearly perfect control of disease at both temperatures. The effects of time and temperature on disease were shown when untreated seed was used.

The control indices for untreated seed following incubations of 0, 5, 10, and 15 days at 4° C. were, respectively, 84.0, 62.7, 0.3, and 0. At 11° C. these were, in the same order, 84.0, 36.3, 0, and 0. These data and the photograph (Fig. 2) show that time of incubation affected the disease level more markedly than did the temperature at which the kernels were incubated. A temperature differential, however, was apparent in the comparison of the 5-day incubations where disease definitely was more severe following incubation at the higher (11° C.) temperature. Other evidence in this experiment that 11° C. was favorable for disease was indicated in a lower control index for treated seed incubated 15 days. Also, the untreated kernels incubated for the longer periods at 11° C. were in a more advanced stage of decay than those incubated for the same periods at 4° C.

DISCUSSION

The reduction in disease when seed was incubated at the lower temperature (4° C.) agrees generally with results reported by Haskell[3] from a similar experiment with sweet corn. He found germination reduced more when seed from inbred lines was incubated at 50° F. (10° C.) than at 40° F. (4.4° C.), and interpreted the deleterious effects, at both temperatures, as due partly to damage from soil fungi and partly to direct damage of the seed by cold. He does not state the basis for his separation of one factor from the other. In the present experiment soil pathogens appeared to be primarily responsible for disease, with cold functioning to retard germination of the kernels, thereby predisposing them to attack by soil fungi. The drop in disease when seed was incubated at 4° C. is attributed chiefly to

[2] Hoppe, Paul E. Differences in Pythium injury to corn seedlings at high and low soil temperatures. Phytopath. 39: 77–84. 1949.

[3] Haskell, Gordon. Effect of low temperature on the germination of inbred lines of sweet corn. Science 107: 150. 1948.

slower growth of the pathogens at this low temperature. Middleton's data[4] (Table 1, pp. 11–12) for mycelial growth of Pythium species on Difco cornmeal agar at various temperatures indicate that growth in this genus is greatly inhibited below 10° C., some species being completely inactive after 24 hours' incubation at 4° C.

The glass-tumbler technique was effective for the study of different aspects of the complicated corn-seedling disease problem. The method should prove useful in ordinary low-temperature germination tests with seed corn

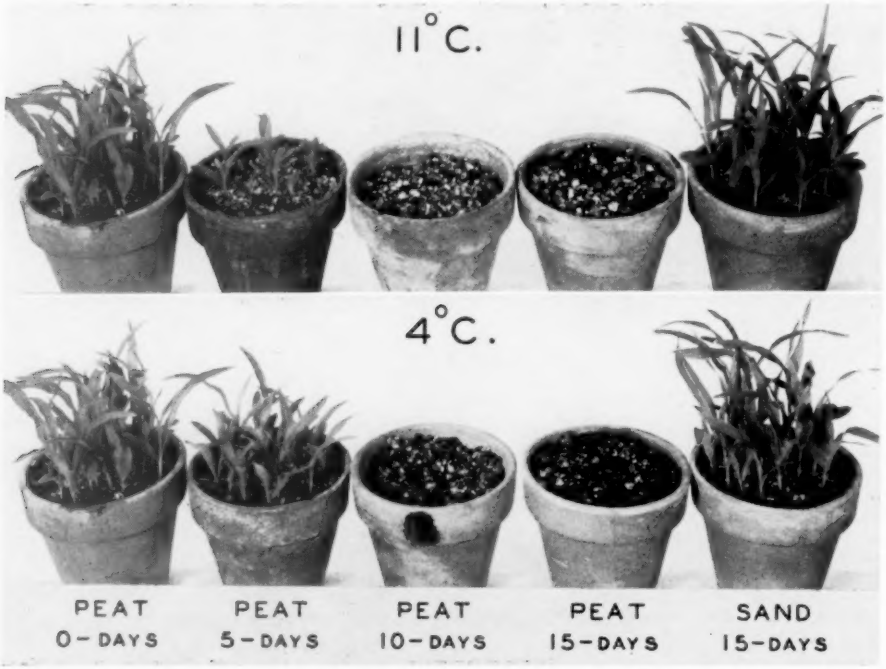

FIG. 2. Effects of length of incubation period (at 4° and 11° C.) of untreated, crown-wounded corn kernels, and the medium in which they were incubated, on the ultimate level of disease in an experiment utilizing the glass-tumbler technique. Note higher incidence of disease at 11° than at 4° C. in peat soil 5 days; absence of disease at either temperature in wet fungus-free sand 15 days.

and also in other special studies. It particularly is adapted for pure culture inoculation experiments and it should greatly facilitate studies of the pathogens that are concerned with seed rots generally, not only in corn, but in other crops as well.

SUMMARY

A new glass-tumbler method permits extensive germination tests with seed corn in cold soil without large refrigeration equipment. The kernels are mixed with wet soil in a glass tumbler and then incubated in an ordinary electric refrigerator at the desired low temperature. Following incu-

[4] Middleton, John T. The taxonomy, host range and geographical distribution of the genus Pythium. Mem. Tor. Bot. Club 20: 1–171. 1943.

bation the kernels are planted in pots and then placed in a warm greenhouse where data are recorded at the 3- to 4-leaf stage of seedling growth.

The glass-tumbler technique gave results similar to those of the method used formerly for testing seed disinfectants for corn in a large walk-in refrigerator. The distinfectants used were Arasan (tetramethylthiuram disulfide 50 per cent), Experimental Fungicide No. 640 (zinc copper chromate), and Experimental Fungicide No. 224 (zinc mercury chromate).

TABLE 1.—*Effects of time, temperature, and medium of incubation on level of disease in crown-wounded corn kernels*

Soil type and seed treatment	Mean control index[a] obtained from seed:						
	Not incubated	Inoculated at					
		4° C. for			11° C. for		
		5 days	10 days	15 days	5 days	10 days	15 days
Sand							
Untreated seed	96.3	91.7	97.7	86.5	99.0	95.5	92.0
Arasan-dusted seed	98.0	94.3	97.3	92.7	97.0	96.3	91.0
Peat soil							
Untreated seed	84.0	62.7	0.3	0.0	36.3	0.0	0.0
Arasan-dusted seed	96.3	93.0	94.0	94.0	92.3	95.7	58.7

[a] Theoretical range of control is from 0 (no seedlings emerged) to 100 (perfect stand of all normal-sized plants). Stunted seedlings were given half the numerical values assigned the healthy plants in calculating the control index.

In a second experiment, ultimate disease effects were compared after untreated and Arasan-dusted, crown-wounded corn kernels had been incubated at 4° and 11° C. for 5-, 10-, and 15-day periods in peat soil and in fungus-free sand. High levels of disease resulted only from incubations of untreated seed in peat soil, in which seed decay reached 100 per cent in incubations of 10 or more days, at either temperature. Disease level was somewhat higher at 11° than at 4° C. following the 5-day incubations. Low temperature in itself caused no injurious effects but served to predispose the slowly germinating kernels to attack by soil fungi.

The glass-tumbler technique may be useful in the study of seed rots and seedling diseases in crops other than corn.

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EFFECT OF TEMPERATURE ON GROWTH AND PATHOGENICITY OF SCLEROTINIA TRIFOLIORUM[1]

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(Accepted for publication March 30, 1951)

Temperature is one of the important factors influencing pathogenicity of *Sclerotinia trifoliorum* Erikss. to Ladino clover and other forage legumes. Since this fungus requires a lower temperature than most common plant pathogens, it is essential to learn the limits within which the organism grows and infects plants before the initiation of a large-scale testing program. According to Pape (7), most cultures of *S. trifoliorum* in Petri dishes containing malt-meat-extract agar grew best at 16.5° C., but two of the six isolates he tested grew best at 19° C. He concluded that the minimum temperature for growth of the fungus was approximately 0° C. and the maximum about 33° C.

Lissitzina (5) reported 21° C. as optimum for mycelial growth of *S. trifoliorum*. According to her investigations, the minimum temperature for development was −2° C. and the maximum 27° C. The fungus was killed at −24° C. and at 42° C. Frandsen (2), Nicolaisen et al. (6), and Rudorf (9) agree with Pape that the optimum temperature for infection approximates 15° C. High relative humidity is also necessary for rapid development of the disease (6, 9).

The occurrence of many biotypes within *S. trifoliorum* has been demonstrated (1, 2, 7, 8). In an earlier study (3) it was shown that different monoascospore cultures varied distinctly in pathogenicity to Ladino clover. This suggested that some isolates might also vary with respect to the temperatures at which they attack plants. Experiments were made to obtain additional information on this point and to determine more precisely the effect of temperature on pathogenicity of *S. trifoliorum*.

MATERIALS AND METHODS

The cultures of *S. trifoliorum* used in all tests reported here were monoascospore isolates obtained from different apothecia collected in fields of diseased Ladino clover in the vicinity of State College, Pennsylvania.

In laboratory tests on rate of growth as affected by temperature, 22 monoascospore isolates obtained from five different apothecia were rejuvenated from stock cultures maintained at 5° C., by growing them on fresh potato-dextrose agar in Petri dishes at 15° C. After 5 days most isolates reached the periphery of a 90-mm. Petri dish. Subtransfers of each isolate were then made by cutting an 8-mm. disk from the parent colony and trans-

[1] Contribution No. 98 of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, State College, Penna., in cooperation with the Northeastern States.

[2] Pathologist and Senior Agronomist, respectively.

The authors are indebted to A. A. Hanson for making the statistical analysis.

ferring it to the center of a fresh Petri dish of potato-dextrose agar. Duplicate cultures of each isolate were grown at 5°, 10°, 15°, 20°, and 25° C. Growth of the colonies was measured daily, but for comparative purposes the amount of growth made by each isolate in 5 days at the different temperatures was used.

Two experiments to determine the effect of temperature on pathogenicity of *S. trifoliorum* to Ladino clover were made in four environmental control chambers in the laboratory. In these chambers, artificial light at 500 foot-candle intensity from "daylight" and "white" fluorescent lamps was regulated to provide a 16-hr. day. Day and night temperatures were kept constant in all tests and relative humidity was 90 per cent or higher except for one chamber in one test at 16° C. and 50 per cent r.h. The Ladino clover plants used in these experiments were from commercial seed sown in steamed, fertile, compost soil in 4-in. glazed crocks. All plants were grown in a greenhouse and transferred to the environmental chambers after inoculation with the fungus.

For the seedling tests, plants were thinned to 10 per pot and inoculated with *S. trifoliorum* when they were approximately 1 month old. For tests with older plants of Ladino clover, seeds were sown in a flat and individual seedlings were transplanted to 4-in. glazed pots and grown in a greenhouse until stolons reached the edge of the pot; those that grew over the edge were pruned back. Plants for each test were selected for uniformity from among a surplus prepared. Each monoascospore isolate of *S. trifoliorum* was tested on four replicate plants of older Ladino clover at each controlled temperature.

Inoculum of each monoascospore isolate was prepared from cultures grown on Pape's wheat-oats mixture for 21 days at 15° C. The infested grain was then dried at room temperature, ground, and stored at 5° C. until used (4). Approximately equal amounts of dried-grain inoculum were applied to the crown and stolons of test plants. The plants were sprayed with distilled water just before they were placed in the temperature-controlled chambers, to moisten the inoculum. Thereafter, the inoculum was moistened daily or oftener if necessary. Noninoculated controls were included at each temperature.

Observations on severity of infection were made every day or two and tabulated numerically on a scale in which 0 represents no damage and 13 represents plants killed. For critical comparisons, the data were analyzed statistically.

Plants were removed from the chambers after they were killed or when the inoculum was spent.

RESULTS

Rate of growth of monoascospore isolates at different temperatures. The rate of growth of the 22 isolates on potato-dextrose agar indicated that a temperature between 15° C. and 20° C. was optimum. The average diam-

eter of the 22 isolates 5 days after incubation at each temperature was 24 mm. at 5° C., 55 mm. at 10° C., 84 mm. at 15° C., 81 mm. at 20° C., and 35 mm. at 25° C. These results agree with those of Pape (7) and others.

Growth was good at 20° C., but this temperature was near the upper limit for development. At 10° C., most cultures grew more slowly and required 7–10 days to reach the periphery of a Petri dish. At 5° C. most cultures required 12–18 days and none of the cultures incubated at 25° C. grew across a Petri dish. Thus, the maximum temperature for growth of the isolates of *S. trifoliorum* tested here was approximately 25° C., and the minimum temperature was probably in the range 0–5° C.

Only two cultures grew more slowly than the others at 15° C. and the optimum temperature for growth of one of these was considerably higher than that of the other. Greatest differences in rate of growth occurred at 25° C., but rates of growth of duplicate plates of the same isolate also fluctuated widely at this temperature. Aside from these exceptions, it was not possible to distinguish culturally between isolates grown at different temperatures.

Temperature range at which S. trifoliorum attacked plants of Ladino clover. For this experiment, seedlings and older plants were inoculated with five monoascospore isolates representing cultures obtained from five different apothecia.

Inoculated seedlings were incubated in the temperature-controlled chambers at 10°, 16°, 21°, and 27° C. Earliest symptoms of infection were drooping leaves and wilted seedlings, within 2 days at 21° C. and within 3 days at 10° and 16° C. Only trace infection developed on plants incubated at 27° C. Maximum damage occurred in 7–10 days and the seedlings were removed from the chambers for final observation 12 days after inoculation.

Damage to seedlings (Table 1) was significantly greater at lower tem-

TABLE 1.—*Comparison of average virulence of different isolates of S. trifoliorum to seedlings of Ladino clover at different controlled temperatures*[a]

Isolate	10°	16°	21°	27°	Mean[b]
1–10	3.9	5.7	6.4	2.1	4.5
2–7	6.0	5.6	10.3	4.1	6.5
5–1	4.2	3.4	5.1	1.4	3.5
6–5	7.2	7.6	9.6	2.9	6.8
7–1	5.7	5.9	10.6	3.0	6.3
Check	0.0	0.0	0.0	0.0	
Mean[c]	5.4	5.6	8.4	2.7	5.5

[a] Figures represent the average of 10 readings on each of four replicate pots based on the index: 0 represents no damage, 13 represents plants killed.
[b] LSD for five isolates (P equals 0.01), 1.30.
[c] LSD for temperatures (P equals 0.01), 1.17.

peratures than at 27° C. Damage at 21° C. was significantly greater than at any other temperature. There was no significant difference in pathogenicity between isolates 2-7, 6-5, and 7-1. These isolates, however, were

significantly more virulent than isolates 1-10 and 5-1. A statistical comparison of seedling survival after attack by the different isolates at different temperatures yielded similar results. At the 5 per cent level, all cultures were significantly more pathogenic than isolate 5-1. There was no significant difference between isolates 1-10, 2-7, and 7-1. Isolate 6-5 was significantly more virulent than isolates 1-10, 5-1, and 7-1, but not 2-7. At the 1 per cent level isolate 6-5 was significantly more pathogenic than isolates 1-10 and 5-1.

The same five monoascospore isolates were used to inoculate older plants of Ladino clover, with the temperature range extended to include 0–1°, 4°, 7°, 10°, 16°, 21°, and 27° C. Because only four chambers were available, the test was made in two parts.

In the first part of the test, one group of plants was inoculated and incubated at 16° C. and at 50 per cent r.h.; another was incubated at the same temperature, but at 90 per cent r.h. The remaining groups of plants were incubated at 21° and 27° C. respectively, and at 90 per cent r.h. In the second part of the test, groups of plants were incubated in a cold room at 0–1°, and in the environmental control chambers at 4°, 7°, 10°, and 16° C. and at 90 per cent r.h.

Greatest infection occurred at 16° and 21° C. and 90 per cent r.h. and only slight to moderate infection at 16° C. and 50 per cent r.h. Infection at 16° and 21° C. was significantly greater than infection at 27° C. At the lower temperatures, older plants were attacked just as severely as seedlings, but it took longer to kill them. While plants incubated at 16° and 21° C. required 9–12 days for maximum infection, those at 4° and 0–1° C. required 17–28 days.

Of the five cultures tested on older plants, isolates 2-7 and 6-5 were more virulent than isolates 1-10, 5-1, and 7-1. Graphs for representative isolates are in figure 1. These differences were significant at the 1 per cent level. Isolates 2-7 and 6-5 did not differ significantly.

Pathogenicity of monoascospore isolates of S. trifoliorum to Ladino clover at different temperatures. For this experiment, the 22 monoascospore isolates cultured in the potato-dextrose agar test were used. These comprised groups of four or five isolates originally obtained from each of five different apothecia and designated as groups 1, 2, 4, 6, and 9. Older plants of Ladino clover were inoculated with each isolate and incubated at 4°, 10°, 18°, and 27° C. Because of limited space it was again necessary to test the plants first at the two lower temperatures and later at the two higher temperatures.

Analysis of variance showed that the effect of temperature (4° in comparison with 10° and 18° C.) was highly significant. Since there was only a trace of infection at 27° C., it was not included in the analysis. The interaction mean squares (temperature × groups and temperature × isolates within groups) were used to compare the response of the groups and isolates within groups for the average of all temperatures. These data showed that

all groups were significantly more virulent than group 9; probably because of the abnormal behavior of one of the isolates comprising this group. Group 6 was significantly more virulent than all others.

For the average of all temperatures, isolates within groups differed significantly in pathogenicity. For example, in group 1, one isolate was significantly more virulent than all others. In group 4, two isolates were significantly more virulent when compared with the least virulent isolate, but were not significantly different from the remaining two. All isolates in group 9 were significantly more virulent than the abnormal isolate.

The interactions of temperature × groups and temperature × isolates within groups were both highly significant.

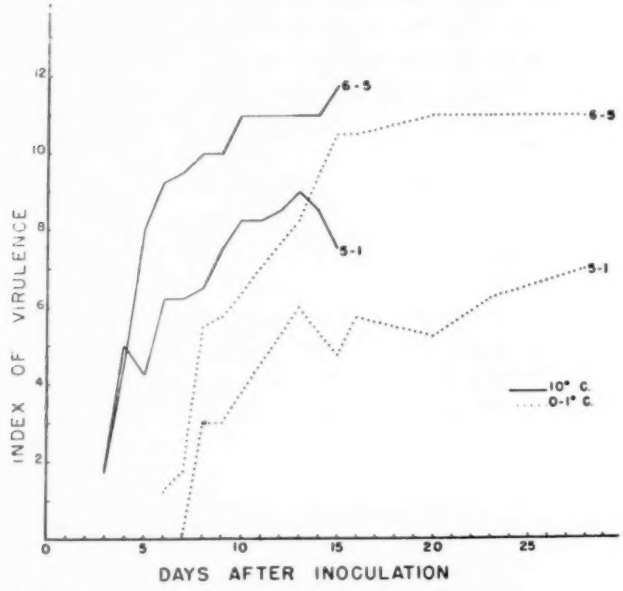

FIG. 1. Comparative virulence of a pathogenic and a less pathogenic monoascospore isolate of *S. trifoliorum* at two controlled temperatures.

When all 22 isolates from the five groups were compared as to total response, they varied considerably in virulence. Nine of the isolates could be grouped in a highly virulent class while five of them constituted a less virulent class.

CONCLUSIONS

From the data presented, it is apparent that *S. trifoliorum* possesses the capacity to grow and attack plants of Ladino clover over a wide range of temperature with the minimum at approximately 0° C. and the maximum between 21 and 27° C. Although it was not possible to distinguish between monoascospore isolates cultured on agar at different temperatures, the isolates differed significantly in pathogenicity at different temperatures when they were tested on seedlings and on older plants of Ladino clover. In

addition, there were highly significant differences in pathogenicity between monoascospore isolates obtained from the same and from different apothecia. These results demonstrate experimentally the broad range of biotypes produced by one of our major forage legume pathogens.

SUMMARY

Best growth of 22 monoascospore isolates of *Sclerotinia trifoliorum* on potato-dextrose agar occurred between 15° and 20° C. The maximum temperature for growth approximated 25° C. and the minimum temperature was in the range 0–5° C. It was not possible to distinguish culturally between isolates grown at different temperatures.

For both seedlings and older plants of Ladino clover, infection at 16° and 21° was significantly greater than at 27° C. Better infection was obtained at 90 per cent than at 50 per cent relative humidity.

There were highly significant differences in pathogenicity between monoascospore isolates obtained from the same and from different apothecia.

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CORN SEEDLINGS AS TEST PLANTS FOR THE SUGAR-CANE MOSAIC VIRUS

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(Accepted for publication April 24, 1951)

The use of sorghum (*Sorghum vulgare* Pers.) as a test plant for the sugar-cane mosaic virus (*Marmor sacchari* Holmes) was mentioned by Smith (4). Adsuar (1) used seedlings of the sorghum variety Hegari to determine some properties of the sugar-cane mosaic virus in Puerto Rico. Seedlings of several varieties of sorghum were employed in tests carried out to compare the properties of the sugar-cane mosaic virus with those of a virus[2] which causes mosaic of the citronella plant (*Cymbopogon nardus* Rendle) in the State of São Paulo, Brazil. Sorghum seedlings were not considered satisfactory test plants under the local conditions because of their slow growth in pots and their narrow leaves, which were difficult to inoculate by rubbing.

The corn plant (*Zea mays* L.) is susceptible to the sugar-cane mosaic virus (2) and grows more satisfactorily in pots than does sorghum. Hence, corn seedlings were tried as a test plant for this virus. Two main sources of corn types were available for study: 1) a collection of standard varieties, including local and introduced types, and 2) the inbred lines being studied locally for the production of hybrid corn seed. The results obtained with corn seedlings of a few inbred lines and with seedlings of some varieties were so satisfactory that the publication of this information[3] was believed to be of interest to workers engaged in experiments with viruses of the same group.

MATERIAL AND METHODS

The inbred corn lines used in the tests were secured from the collection maintained by the Genetics Department, Instituto Agronómico de Campinas, Brazil. They have been selfed for 18 generations and show a high degree of homozygosis. The varieties were received from the same source.

Corn seedlings were raised in 6-in. pots, four or five plants in each pot. They were usually 8–15 days old when inoculated. At this age the seedlings were about 5 in. high and had two leaves that could be inoculated by rubbing.

The sugar-cane mosaic virus was secured from plants of the sugar-cane variety P.O.J. 213, affected with the type of mosaic commonly found in Campinas, São Paulo. The citronella mosaic virus was also obtained from citronella plants grown in the same region.

Inoculum for the tests was prepared by grinding leaves of affected plants in a meat grinder, and then straining the ground pulp through a double

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[2] This virus is now considered to be a strain of the sugar-cane mosaic virus complex.

[3] A short note on this subject is scheduled to appear in Bragantia, a research journal published by the Instituto Agronómico de Campinas, Brazil.

layer of muslin. The infectious juice thus obtained was used as such or diluted 1 : 5 with 0.01M sodium sulphite. Carborundum powder (600-mesh) was sprinkled on the leaves of test plants prior to inoculation or added to the inoculum. The inoculation was done by rubbing two or three leaves of each seedling, usually three strokes per leaf, with a small pad of muslin moistened in the inoculum.

SUSCEPTIBILITY OF INBRED LINES AND CORN VARIETIES TO THE SUGAR-CANE AND CITRONELLA MOSAIC VIRUSES

A first test was made with 35 inbred lines derived from four local corn varieties. In this test eight plants of each line were inoculated with the sugar-cane mosaic virus and eight plants with the citronella mosaic virus; eight plants remained uninoculated, as controls. More than half of the lines tested were susceptible to both viruses, although the majority of these were not susceptible enough to rate as good test plants. A few lines—419 from the variety Cateto, 2073–1–1–1 from Armour, and 718 and 754 from the variety Cristal—gave practically 100 per cent infection with both viruses, and proved to be very good test plants. A few lines had no symptoms.

Symptoms in infected seedlings usually appeared, 4 to 8 days after inoculation, as small chlorotic or pale dots in linear arrangement in the middle or at the base of a young unfolding leaf. These dots increased in size and coalesced. At a later stage these leaves showed typical mosaic in which the light green and normal green areas occurred mostly in a longitudinal pattern.

No consistent difference in symptoms could be noticed between plants of the same line inoculated with the sugar-cane and the citronella mosaic viruses. There were, however, differences in the symptoms shown by seedlings of different lines. Seedlings of line No. 419 showed early, conspicuous symptoms (Fig. 1) which remained so as the plants grew. In other lines symptoms were more visible after infection and decreased in intensity later. There was also some variation in the time taken for symptoms to appear. Line No. 754, besides being highly susceptible, showed symptoms usually 4–5 days after inoculation, whereas other lines required one or two days longer for first symptoms to appear.

In tests with corn varieties, 10 plants of each variety were inoculated with the sugar-cane mosaic virus and 10 plants with the citronella mosaic virus; 10 plants were kept as checks. Of 113 varieties thus tested the great majority showed practically 100 per cent infection when inoculated with either virus. The varieties listed below were highly susceptible and showed the symptoms more readily. Any of these could be used as a test plant for the sugar-cane mosaic virus: 372, County White Dent; 373, Itaici; 391, Funk; 440, Prolifics; 936, Hybrid KYS × 5120; 948, White Station Mosby; 1082, Michelson's Giant Yellow Dent; 1149, Palha Inteira; and 1223, B8. The symptoms induced on different corn varieties by the sugar-cane and by

the citronella mosaic viruses were similar. Symptoms on most varieties began to appear 5 days after inoculation, and did not differ from those shown by seedlings of inbred lines.

FIG. 1. A. Corn seedlings of inbred line 419: a, plants infected with the sugar-cane mosaic virus, 10 days after inoculation; b, healthy controls. B. Leaves from plants of inbred line 419: a, infected by the sugar-cane mosaic virus; b, infected by the citronella mosaic virus; c, healthy control.

PROPERTIES OF THE SUGAR-CANE MOSAIC VIRUS

The experiments indicated that corn seedlings were highly sensitive to the sugar-cane mosaic virus. Such seedlings were then used as test plants to determine some of the physical properties of this virus.

Tests on aging *in vitro* and on dilution were made with infectious juice

obtained from sugar-cane plants. The diluent was an 0.01M solution of sodium sulphite. In tests on thermal inactivation, sugar-cane mosaic virus from infected corn plants was used.

The results obtained in the tests on physical properties are in table 1. The sugar-cane mosaic virus was infectious at dilutions of 10^{-3} and some infection was obtained at dilutions as high as 10^{-4} and 10^{-5}. Infectivity of the undiluted plant extract was greatly reduced by heating at 50° C. for 10 min., but some infection was still obtained at 55° C. The sugar-cane mosaic virus remained active in undiluted plant extract for 24 hr.

TABLE 1.—*Physical properties of the sugar-cane mosaic virus as determined with corn seedlings*

Physical property and procedure	Number of plants inoculated	Percentage of plants infected
Dilution end point		
Undiluted	30[a]	93.3
10^{-1}		95.0
10^{-2}		66.0
10^{-3}		26.7
10^{-4}		6.6
10^{-5}		3.3
Thermal inactivation		
Not heated	30	100.0
50° C., 10 min.		16.7
55° C., do		3.3
60° C., do		0.0
65° C., do		0.0
Aging *in vitro*		
0 hr.	20	95.0
5 hr.		95.0
7 hr.		95.0
9 hr.		65.0
24 hr.		30.0
48 hr.		0.0

[a] For the 10^{-1} dilution only 20 plants were inoculated.

INFECTIVITY OF PLANT EXTRACT FROM INFECTED CORN AND SUGAR-CANE PLANTS

In breeding new varieties of sugar cane resistant to mosaic, large amounts of inoculum are required to inoculate thousands of seedlings in order to select those which are resistant. The leaves of the sugar-cane plant produce little extract and its tissues are relatively difficult to grind. On the other hand, corn plants give more juice than do sugar-cane leaves, and corn tissues are easier to grind. It would be advantageous in breeding projects if corn plants could be used as a source of virus to inoculate sugar-cane seedlings. This would make it possible to obtain not only large amounts of inoculum within a short time, but also inoculum of the various strains desired. Hence, tests were made to determine whether the extract from corn plants infected with the sugar-cane mosaic virus was as infectious as that obtained from diseased sugar-cane field plants. In a first comparison a dilution series of both leaf extracts was tested on corn seedlings. In another test a standard

dilution of plant extract from corn and from sugar-cane plants was compared by inoculation on a number of sugar-cane seedlings from various crosses.[4] The results are in tables 2 and 3. The extract obtained from infected corn plants was slightly more infectious than that obtained from diseased sugar-cane plants. Very good results were obtained in other tests with inoculum of sugar-cane mosaic virus from corn used on several thou-

TABLE 2.—*Results of tests comparing various dilutions of sugar-cane mosaic virus in extract from sugar-cane and corn leaves by inoculation on corn seedlings*

Dilution of inoculum	Leaf extract from	Number of corn seedlings infected of 15 inoculated
Undiluted	Sugar cane	13
	Corn	15
10^{-1}	Sugar cane	14
	Corn	15
10^{-2}	Sugar cane	9
	Corn	12
10^{-3}	Sugar cane	3
	Corn	7
10^{-4}	Sugar cane	0
	Corn	1

TABLE 3.—*Comparative inoculation of sugar-cane seedlings with sugar-cane mosaic virus from corn and from sugar-cane plants*

Parentage of seedlings	Number of seedlings inoculated with each inoculum	Percentage infection with virus from	
		Sugar cane	Corn
Co. 313 × Co. 285	61	70.5	96.7
Co. 421 × CP 11–65	54	29.6	57.4
CP 28–9 × CP 27–108	9	33.3	33.3
Co. 419 × CP 11–65	9	11.1	22.2
Co. 312 × Co. 285	8	100.0	100.0
Co. 312 × US 1694	8	62.5	87.5

sand sugar-cane seedlings during the latter part of 1950. The use of inoculum from corn is now a standard procedure in the sugar-cane breeding project at the Instituto Agronómico de Campinas.

SUMMARY AND CONCLUSIONS

Corn seedlings of some inbred lines and varieties are excellent test plants for the sugar-cane mosaic virus. Eight- to 12-day-old seedlings usually show 100 per cent infection when rubbed with infectious juice plus carborundum. Symptoms of infection may appear, as soon as 4 days after inoculation, as small chlorotic dots arranged linearly in the middle or base of an unfolding leaf. Corn plants grow better in pots than do sorghum seedlings, and their leaves are better for mechanical inoculation. Further-

[4] The authors are indebted to Dr. J. M. Aguirre, Head, Sugarcane Division, Instituto Agronómico, for supplying the sugar-cane seedlings used in these tests.

more, the use of inbred lines with a high degree of homozygosis insures uniform results in the tests.

Physical properties of the sugar-cane mosaic virus were determined on corn seedlings. The virus was active in inoculum heated at 55° C. for 10 min., and in inoculum aged for 24 hr. at room temperature. Infectivity was greatly reduced by dilution at 10^{-3}, but infection was obtained in a few cases with inoculum diluted at 10^{-4} and 10^{-5}. These results are slightly different from those obtained by previous authors (1, 3) and are interpreted as being due to a higher susceptibility of corn seedlings to the virus in comparison with sorghum seedlings and sugar-cane plants which were used by them as test plants.

Extract from corn plants recently infected with the sugar-cane mosaic virus was found to be equally infective or more so than juice of leaves from diseased sugar-cane field plants. The use of inoculum from corn plants is considered advantageous in inoculating sugar-cane seedlings in breeding projects.

Determination of the various strains of the sugar-cane mosaic virus has been made by the reaction induced on a number of sugar-cane varieties (5). Inoculation of individual strains on a number of corn lines and varieties might disclose differences that would permit the use of corn types as differential hosts for the strains. It is not inconceivable that some types of corn might even react to the virus with local lesions.

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PHYTOPATHOLOGICAL NOTES

Longevity of the Red-Node Virus in Beans.[1] W. D. THOMAS, JR., AND R. W. GRAHAM. It has been reported that the red-node virus of bean loses its infectivity within 48 hr. at 22° C. *in vitro* and in dry tissue after 30 days.[2] However, during recent field studies it was suspected, on several occasions, that the red-node virus was being transmitted by the cultivator blades from the remains of infected plants in the soil.

To check the validity of this suspicion, four sets of 12 plants each in six replications were treated as follows: one set was inoculated with extracts from infected vines which had been air-dried and kept at 30° C. for 7 months. This material was soaked in water prior to extraction. A second set was inoculated with extracts from infected vines which had been frozen in moist soil, stored for 7 months, and thawed for 24 hr. at the end of 2, 4, 6, and 7 months. For a third set quick-frozen infected vines which had been kept frozen during the 7-month period were used. The fourth set was a noninoculated check.

Water was used in extracting inoculum only from the air-dried series. For inoculum from the frozen material, juice was expressed from the frozen plants. No buffer reagent was added prior to inoculation.

All plants were kept in insect-proof wire-mesh cages from the time of emergence to prevent chance inoculation. The susceptible pinto bean variety University of Idaho No. 78 was used throughout this test. Inoculations were made on both primary leaves as soon as they had unfolded, using No. 320 carborundum powder as an abrasive. The number of lesions on the 12 plants in each replicate were recorded, together with notes on the severity of infection 30 days after inoculation.

Some infective virus apparently was present in the dried vine material used in the first series, since an average of 8.2 lesions appeared 18 days after inoculation; however, these lesions showed little tendency to merge or become systemic. The second series, in which dried material had been successively frozen and thawed, showed significantly more infection, with an average of 17.8 lesions within 13 days after inoculation; yet few of the lesions merged or passed into the petioles. In the third series an average of 25.2 lesions resulted from inoculation with frozen material; the lesions developed within 5 days after inoculation and spread into the petioles and nodes of the test plants. Within 30 days several of the plants were near death. These results are highly significant over the results obtained with dry tissue, but are not significant over those with thawed material. The checks showed no evidence of infection. The reason for the difference in time necessary for symptoms to appear is not understood; however, fresh extracts of the red-node virus seem to cause production of symptoms by

[1] Published with the approval of the Director, Colorado Agricultural Experiment Station, as Scientific Journal Series No. 352.

[2] Thomas, H. Rex, and W. J. Zaumeyer. Red node, a virus disease of beans. Phytopath. 40: 832–846. 1950.

host plants sooner than do older extracts. Similar results were obtained on repetition of the experiment. The accumulated data show that the red-node virus may remain active at least 7 months in dried or frozen bean tissue. It is possible that such infected material may be a source from which the virus is transmitted by mechanical means to plants of a later crop.—Department of Botany and Plant Pathology, Colorado A & M College, Fort Collins, Colorado.

Bronze Leaf of Coconut Palm in the Dominican Republic and Venezuela. R. CIFERRI. Bronze leaf of the coconut palm has been observed by the writer in the Dominican Republic since 1931–1932, but has been confused with either red ring or bud rot. The presence of the disease near San Cristobal, Dominican Republic, was confirmed during a trip along the south Caribbean coast in November 1946.

In regard to the controversy over the identity of bronze-leaf disease or bronze-leaf wilt in Jamaica and Trinidad, the symptomatology and succession of symptoms are similar to those described by Leach[1] from Haiti. An interesting observation is the presence, in the same southern province, of a disease on royal palm (*Roystonea regia* s. lato; = *R. hispaniolensis*) related or identical to that observed on date palm in Haiti. A more detailed study of the disease on both palms is needed.

In view of the dispute as to the identity of the two diseases, the occurrence of bronze leaf of coconut palm in Venezuela is interesting because of that country's intermediate geographical position between Jamaica and Trinidad Islands.

Observations made along the south Caribbean coast demonstrated that the symptomatology, on the basis of descriptions by Leach,[1] is partly of the Trinidad type and partly of the Jamaica type.

Two interpretations are possible: a) one and the same disease occurs throughout the West Indies, as stated by several workers,[2, 3, 4, 5, 6] prior to Leach's observations; or, b) both the Jamaica and Trinidad diseases are present in Venezuela. A differential or comparative study of the symptomatology of the disease in Venezuela, starting from the Maracaibo coast (or, better, from the western Colombia coast where the same disease is present[7]) to the Paria Peninsula near Trinidad, may resolve the problem.

In Venezuela the disease occurs irregularly and in discontinuous areas,

[1] Leach, R. The unknown disease of the coconut palm in Jamaica. Trop. Agr. 23 (3): 50–56. 1946.

[2] Bain, F. M. Bronze leaf wilt disease of the coconut palm. 48 pp. Trinidad Govt. Printing Office. 1937.

[3] Bain, F. M. Report on the coconut growing areas of Jamaica. Bul. Dept. Agr. Sci. Agr. Jamaica N. S. 22. 1940.

[4] Bain, F. M. Coconut growing areas of Jamaica. The Port-of-Spain Gazette, Nov. 12, 1949. Roots and drought. Ibid., Nov. 13, 1949. Great deal yet to be done from academic standpoint. Ibid., Nov. 14, 1949.

[5] Briton-Jones, H. R. Wilt diseases of coconut palms in Trinidad. Part I. Suppl. to Trop. Agr. 5 (5): 1928. Part II. Suppl. to Trop. Agr. 6 (12): 1929.

[6] Martyn, E. B. Coconut diseases in Jamaica. Part I. Trop. Agr. 22 (3): 51–59. Part II. Trop. Agr. 22 (4): 69–76. 1945.

[7] A personal observation in 1948, confirmed by Dr. F. M. Bain.

with healthy plantings in isolated valleys and more or less continuous belts along open coasts. The disease is largely independent of edaphic and climatic conditions, appearing in sand, alluvial, loam, or half-clay soils with alkaline, neutral, or slightly acid reaction, in belts from very arid to moist regions. It occurs on irrigated coconut plantations (e.g., in Cata Valley, State of Aragua), and even along irrigation canals.

In the author's opinion, physiological drought is a consequence of the disease more than a causative factor. Bronze leaf appears to be an endemic and locally slowly spreading disease with occasional exceptionally rapid means of dissemination; in any case, like a disease incited by a living agent. Is it merely a coincidence that, as planters affirm, the disease appears after hurricanes—e.g., in San Cristobal just after 1930, in Paria Peninsula after 1933, and in Jamaica after the years 1932 and 1933?

The sequence in development of symptoms is obscured by the heterogeneity of coconut races that react differently under culture.—Italian Cryptogamic Laboratory and Botanical Institute, The University of Pavia, Italy.

Red Rot of Sisal in Venezuela. R. CIFERRI. A destructive disease of sisal (usually classified as *Agave rigida* var. *sisalana*, with forms), was found in Venezuela in 1948 and, apparently, is the same disease formerly observed by the writer in the northwestern part of the Dominican Republic, W. I., on the same host plant as well as on henequén (*Agave fourcroides*). In Venezuela, this disease is apparently localized in the Barquisimeto region, the greatest center of sisal culture of the Republic.

Red rot is characterized by a gradual, basal discoloration beginning with the bud leaves and then spreading upward to the basal leaves. After one or more seasons, the buds are affected by a soft rot so that when the bud is pulled the rosette easily separates from the subterranean stem, leaving only the old peripheral foliar crown attached to the plant. An early sign of the disease is the cinnabar-red stain in the stem and the leaf blades.

The disease does not appear to spread rapidly from plant to plant. However, it is readily spread by the use of suckers from diseased plants for new plantings. As a result, fields have been found with 90 per cent or more of the plants infected.

A number of nonpathogenic bacteria and a few saprophytic fungi may be isolated from rotted portions of the host plant but none from the red-stained, nonrotted tissues. A rot which is probably not the same as red rot has been reproduced by inoculating small fragments of the stained tissues into healthy ones. This experiment, however, was not carried on under perfectly aseptic conditions.

The red rot disease has been introduced from Barquisimeto to a second sisal center of Venezuela, namely, Margarita Island (Nueva Esparta), and the fields of sisal near Pampatal have been practically destroyed from 1947 to the present. The disease has not been found in the fields at El Cardon on the same island.

Red rot is clearly distinct from potassium deficiency[1] and from the basal leaf necrosis found in Brazil,[2] as well as from the leaf disease of Tanganyika[3] and the mosaic of Belgian Congo.[4] The last, which, in common with red rot, has internal red stains during the last stage of development, has been attributed both to a virus and to a nutrient deficiency.

The etiology of red rot of sisal is a matter of dispute. In our opinion it cannot be considered a deficiency disease because it was introduced into Margarita Island by suckers from Barquisimeto; and because the incidence of the disease is reduced by multiplying sisal plants with suckers from mother plants free from the disease. Cutting the foliar rosette of the mother plant before picking the suckers also helps reduce the incidence of the disease. Finally, the fact that the disease develops on different soil types would tend to discredit the theory that red rot is a deficiency disease. A working hypothesis is, then, that the disease is caused by a virus.

In working with red rot it was found that *Colletotrichum agaves* Cav. and *Marssonina agaves* Earle are present in Venezuelan sisal fields but that they are of little economic importance.—Italian Cryptogamic Laboratory and Botanical Institute, The University of Pavia, Italy.

[1] Da Silva Fernandes, C. O rolamento do broto do Sisal (Agave sisalana Perrine). Bol. S. A. I. C. 11: 60–63. 1944.

[2] Medina, J. C. "Leaf basal necrosis" of sisal (English 81). Bragantia, S. Paulo 3: 73–84. 1943.

[3] Morstatt, H. Weiteres über Blattkrankheiten der Sisalagave. Der Tropenpfl. 34: 5–13. 1931.

[4] Staner, P., and G. Verplaneke. Étude d'un état pathologique du Sisal au Congo Belge. Bul. Inst. Roy. Colon. Belge 1: 279–300. 1930.